Vol. 35, No. 1 March 1948

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN
J. W. EVANS
D. J. FINNEY
H. MARTIN

CAMBRIDGE UNIVERSITY PRESS
CAMBRIDGE, AND BENTLEY HOUSE, LONDON
AGENTS FOR U.S.A.
THE UNIVERSITY OF CHICAGO PRESS
CANADA AND INDIA: MACMILLAN

CONTENTS OF VOL. 35, NO. 1

ERRATUM

The Annals of Applied Biology, **34** (3), p. 463

Add to List of Exhibits at Conversazione on 25 April 1947:

Photomicrographs and drawings showing: the flagellate origin of malarial trophozoites from exflagellated chromidia in Grassi's prognosticated developmental arc between sporozoites and trophozoites: the persistence of sporozoites; pleomorphism of human malaria parasites. MR K. B. WILLIAMSON

VOLUME 35, NO. 1 MARCH 1948

STUDIES ON THE RELATIONSHIPS BETWEEN EARTHWORMS AND SOIL FERTILITY

II. SOME EFFECTS OF EARTHWORMS ON SOIL STRUCTURE

BY A. C. EVANS

Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts

(With 1 Text-figure)

The weight of wormcasts thrown on to the surface of eight fields of differing agricultural history depends on the numbers of *Allolobophora longa* Ude and *A. nocturna* Evans present and also on the mean size of the individuals of these two species. The weight of wormcasts produced per acre per annum on the different fields varied from 1 to 25 tons and it is calculated that from 4 to 36 tons of soil per acre per annum pass through the alimentary tracts of the total population of earthworms present. The percentage pore space of a soil containing a high population of wormcasting species is much greater than that of a field with a high population of worms which do not produce wormcasts. In two old pastures, the amount of coarse sand relative to silt and clay was found to increase appreciably with depth: this distribution is probably a result of the long-continued activity of earthworms.

THE EFFECT OF DIFFERENT EARTHWORM POPULATIONS ON WORMCAST PRODUCTION

Evans & Guild (1947) have shown that, on a permanent pasture field at Rothamsted, the weight of soil brought to the surface by earthworms for two successive years, 1944–5 and 1945–6, was 11·5 and 11·0 tons/acre respectively. They also showed that the fluctuations in the weight of wormcasts brought to the surface during 4-day intervals were more closely correlated with the numbers of active *Allolobophora nocturna* Evans and *A. longa* Ude than with the total number of active worms. Also, field surveys on the Rothamsted Farm showed that the numbers and proportions of the different species of earthworms present in a field depended chiefly upon the previous agricultural history of the field. Advantage was taken of the knowledge thus gained to study the effects of different earthworm faunas upon the rate of production of wormcasts and the consequent sinking of stones and formation of the layer of vegetable mould, and upon the particle size distribution of the soil in the different layers.

Eight fields were chosen for this investigation, six under grass and two in arable cultivation. The selected fields show a wide variation in their agricultural history.

Parklands. Very old grass; until a few years ago a large number of deciduous trees were present but these have gradually been cleared during the last 4 years.

Great Field III. Put down to grass about 1870.

Great Field II. Second year grass following 2 years arable; previously as Great Field III.

Pastures. Seventh year in grass after many years arable.

Claycroft. Fourth year in grass after larch and spruce, felled in 1939 and reclaimed in 1942.

New Zealand. First year in grass following 2 years arable, previously grass put down in 1928.

Stackyard. In stubble after spring wheat, previously grass put down in 1928.

Delharding. In stubble after winter oats, fifth cereal crop after old grass.

Seven circular plots, each 7 sq.ft. in area, were marked at random in each field except in Stackyard where the area available for sampling was only about one-sixth of the field, but visual examination suggested that the wormcast production of the rest of the field was not different from that sampled. The grass on the pasture field plots and the weeds and stubble on the arable field plots were cut on 15 and 16 October 1946 and plots cleared of existing casts. The casts produced in the succeeding week were counted, collected, dried at 105° C., and weighed. As soon as the wormcasts were collected, or at most the day after, the plots were watered with a dilute solution of potassium permanganate to obtain an estimate of the number and species of worms present on the plots. The weight of worms per sample was also determined but after preservation for several days in 5% formalin.

Analyses of variance carried out on a square-root transformation of the numbers and weight of wormcasts per plot show highly significant differences between the various fields (Table 1).

TABLE 1. *The effect of the previous agricultural history of a field on the production of wormcasts*

Mean weights per plot in g.			Mean numbers per plot		
Field	Observed	√	Field	Observed	√
Parklands	232·4	15·07	Parklands	56·6	7·49
Stackyard	110·1	10·10	Great Field III	38·6	6·14
Great Field III	104·0	10·01	Great Field II	28·3	5·23
Great Field II	56·5	7·39	Stackyard	26·7	5·12
Delharding	26·8	4·56	Delharding	13·7	3·37
Pastures	21·1	4·22	Pastures	10·6	3·14
Claycroft	18·5	3·40	Claycroft	8·7	2·49
New Zealand	9·0	2·81	New Zealand	7·0	2·48
Standard error of difference	—	±1·23		—	±0·62

In regard to the weights of wormcasts per plot, the fields fall into four distinct groups. The first group consists of one field only, Parklands, which is the oldest pasture field examined. The second group consists of two fields, one permanent pasture for about 75 years, the other in its first arable year but having had 17 years under grass. Group three consists of one pasture field, Great Field II, with a long previous history of grass but recently broken by 2 years arable cultivation. The fourth group consists of four fields, one (Delharding) having had the last 5 years under

arable cultivation and three being comparatively young pastures. The grouping of the fields for numbers of wormcasts is very similar to that for weight of wormcasts except that groups two and three on the weight basis fall into one group on the number basis. The results clearly show that a long period under permanent grass is favourable to wormcast production but that continuous arable cultivation is unfavourable. This is particularly well shown by three fields which were put down to grass about 1870. Great Field III, undisturbed grass, yielded 104 g. of casts per plot; Great Field II, 2 years arable interruption, yielded 56 g./plot, and Delharding, 5 years continuous arable, yielded only 27 g./plot. Thus 5 years arable cultivation reduced wormcast production by 75%. New Zealand and Stackyard were put down to grass in 1928; the first year of arable cultivation does not appear to have had a marked effect on the production of wormcasts on Stackyard, the yield, 110 g./plot, being very similar to that of Great Field III, but the yield, 9 g./plot on New Zealand after 2 years arable seems to be extremely low compared with the effect of 5 years arable on Delharding.

Information is available on the numbers, species and weights of worms present in the wormcast plots of the eight fields studied. The data relevant to the present discussion is given in Table 2, where the order of the fields follows that of the weight of wormcasts per plot.

TABLE 2. *Mean numbers of all worms and of* A. nocturna + A. longa *and mean weight (g.) of all worms per sample*

	Numbers				Weight (all worms)	
	All worms		*A. nocturna* + *A. longa*			
	Observed	√	Observed	√	Observed	√
Parklands	119	10·9	52	7·2	64·3	8·0
Stackyard	47	6·8	19	4·3	36·6	5·8
Great Field III	47	6·7	21	4·5	39·5	6·2
Great Field II	49	6·9	14	3·6	28·5	5·3
Delharding	24	4·7	4	1·7	8·8	2·8
Pastures	56	7·4	5	2·2	48·0	6·8
Claycroft	38	6·1	8	2·7	47·1	6·5
New Zealand	28	5·1	4	1·8	11·1	3·2
Standard error of difference	—	±0·64	—	±0·57	—	±0·69

Clearly there are highly significant differences between the fields for all three variables. A detailed discussion of these differences will be dealt with in another paper as further information is available for fields from which no collections of wormcasts have been made. It can be seen, from Table 2, that fields having a high production of wormcasts, Parklands, Stackyard and Great Field III, have high populations of all worms but that fields having high populations of all worms do not necessarily have a high wormcast production, i.e. Great Field II and Pastures. The same generalization holds between sample weights of worms and wormcast

production. In the case of the sum of *A. nocturna* plus *A. longa* and wormcast production, it can be seen that fields which have a high wormcast production have a high population of these two species but that fields with a low wormcast production have low populations.

These relationships were further investigated statistically by means of correlation coefficients and analysis of covariance, again carried out on a square-root transformation of the data.

TABLE 3. *Correlation coefficients*

	Total	Within fields	Between fields
Numbers of all worms and numbers of wormcasts	0·693	0·498	0·770
Numbers of all worms and weight of wormcasts	0·695	0·568	0·741
Numbers of *A. nocturna*+*A. longa* and numbers of wormcasts	0·881	0·742	0·932
Numbers of *A. nocturna*+*A. longa* and weight of wormcasts	0·881	0·613	0·962
Weight of all worms and weight of wormcasts	0·519	0·507	0·530
Numbers of all worms and numbers of *A. nocturna*+*A. longa*	0·808	0·593	0·888
$P=0·05$	0·262	0·304	0·707
$P=0·01$	0·340	0·393	0·834

Only one coefficient is non-significant, that between fields for weight of all worms and weight of wormcasts, two are significant at the 5% level, i.e. numbers of all worms with both numbers and weights of wormcasts between fields. All the others are highly significant. The correlation coefficients between wormcasts and numbers of *A. nocturna* and *A. longa* are in all cases higher than the corresponding coefficients between wormcasts and numbers of all worms. This suggests that the correlations between wormcasts and numbers of all worms are largely, if not wholly, due to the proportion of *A. nocturna* and *A. longa* present.

TABLE 4. *Partial correlation coefficients between numbers of all worms and wormcasts*

	Total	Within fields	Between fields
Numbers of all worms and numbers of wormcasts	−0·069	0·107	−0·224
Numbers of all worms and weight of wormcasts	−0·059	0·321	−0·898
$P=0·05$	0·329	0·341	0·836
$P=0·01$	0·401	0·432	0·917

Calculation of the partial correlation coefficients (Table 4), shows that all the correlation between numbers of all worms and wormcasts is due to the presence of *A. nocturna* and *A. longa*, the only significant correlation being negative. A similar result was obtained by Evans & Guild (1947) for the correlation between wormcast production and numbers of worms other than *A. nocturna* and *A. longa* during the period May 1945 to April 1946.

In view of the highly significant correlations between wormcast production and numbers of *A. nocturna* and *A. longa* it is possible to test whether factors other than

the number of these two species present in a field have any effect on wormcast production. This can be done by adjusting the field means to a constant *A. nocturna* and *A. longa* population, by means of the within fields regression coefficient, Snedecor (1946). The adjusted field means, together with the appropriate standard error, are shown in Table 5.

TABLE 5. *Mean wormcast production per plot adjusted for differences in numbers of* A. nocturna *and* A. longa. (*Square-root transformation*)

Field	Wormcast production in g.	Numbers of wormcasts
Parklands	10·18	4·51
Stackyard	9·07	4·50
Great Field III	8·67	5·32
Great Field II	7·30	5·17
Delharding	6·89	4·79
Pastures	5·91	4·17
New Zealand	5·08	3·86
Claycroft	4·47	3·14
Standard error of difference of adjusted mean	±0·99	±0·83

The order of the adjusted and actual mean weight of wormcast production has scarcely changed. There are still significant differences between the wormcast production on certain fields after allowing for the effects of differing populations of *A. nocturna* and *A. longa*. Parklands, Stackyard and Great Field III have a significantly higher wormcast production than Pastures, New Zealand and Claycroft. Sufficient data are not available to determine the reason for these significant differences in adjusted means but it is reasonable to suppose that the average size of the worms on a field will influence cast production. This proved to be the case. Figures are available for the mean weight of individuals of *A. nocturna* and *A. longa* on the eight fields and their correlation with wormcast production is highly significant but with a negative sign, $r = -0{\cdot}888$, $P = < 0{\cdot}01$. The negative sign of the coefficient shows, unexpectedly, that small worms produce a greater weight of casting than an equal weight of larger worms, i.e. growing worms are more active relatively than adults. Unfortunately, the figures on the mean weight of worms relate only to differences between fields, none being available to estimate variation within fields, and so it is not possible to calculate whether this factor would account for the remaining significant differences between fields after eliminating the effects of varying populations.

Since the fields selected for this study differed considerably in their previous agricultural history, particularly in the time since last ploughed, it was expected that soil factors, such as state of consolidation, might have an effect on the wormcast production. This does not appear to be the case. Stackyard, ploughed 7 months before the casts were collected, is second, and Delharding, arable for 5 years, is fifth in order of production for both unadjusted means and means adjusted for population differences.

The variation in the mean numbers of wormcasts on the eight fields does, however, seem to be due only to the variation in the numbers of *A. nocturna* and *A. longa* present. The adjusted mean number of wormcasts present on Claycroft is significantly lower than those on Great Fields II and III, while the other significant differences shown in Table 1 have disappeared.

It would seem therefore in the circumstances of the present study that differences in the production of wormcasts on fields of varying agricultural history are chiefly, if not wholly, due to differences in the populations of only two of the several species of earthworms present.

WEIGHT OF WORMCASTS PRODUCED PER ACRE

From the data obtained in the present study and that of Evans & Guild (1947) it is possible, making certain assumptions, to calculate the approximate minimum weight of soil which annually passes through the alimentary canals of the earthworm population of the several fields. Evans & Guild found for two successive periods of 12 months on Great Field III that the production of wormcasts was 11·5 and 11 tons/acre. The time of collection of wormcasts in the present study was arranged so that it occurred when soil conditions were very favourable, i.e. moist and warm. Similar conditions occurred at the end of October and beginning of November 1945 and a comparison of wormcast production and numbers of *A. nocturna* and *A. longa* per seven plots at that time and October 1946 are given below:

	1945	1946
Wormcast production	799 g.	728 g.
Numbers of casts	253	270
Number of worms	505	474
Numbers of *A. nocturna* and *A. longa*	190	210

The figures for the 2 years are very similar so the assumption can be made that 728 g. of wormcasts collected in 1946 represents approximately an annual production of 11 tons/acre. On this basis the weights of wormcasts collected on the various fields can be converted to tons per acre per annum.

It is reasonably certain that the production of wormcasts is due to the activities of two species only, the remaining species voiding the soil which they ingest into spaces occurring below the soil surface. If it is assumed that all the soil ingested by the wormcasting species is ejected on to the surface and that, weight for weight, the other species consume an equal amount of soil but void it underground then, knowing the weights of the two groups, a simple calculation gives the weight of soil consumed by the non-casting species. Table 6 gives the results of these calculations.

The weight of soil thrown up on to the soil surface does not represent the total amount of soil consumed by the wormcasting species since some of the soil swallowed is used to line the burrows which they construct in the subsoil. Also during frosty spells in winter, cast production is very meagre or nil because the worms have retreated into the lower, warmer layers of the soil and presumably void ingested soil

there. The figures given in Table 6 therefore represent a minimum turnover of the soil by earthworms.

TABLE 6. *Weight of soil, in tons per acre per annum, either ejected on to the surface of the soil or voided beneath the surface*

Field	Weight of wormcasts	Weight voided beneath the surface	Total soil consumption
Parklands	24·6	11·7	36·3
Pastures	2·3	20·6	22·9
Stackyard	11·6	9·6	21·2
Great Field III	11·0	8·6	19·6
Claycroft	2·0	17·1	19·1
Great Field II	6·0	8·1	14·1
Delharding	2·8	2·0	4·8
New Zealand	1·0	2·7	3·7

The wormcast production on the different fields varies considerably, from 1 to 25 tons/acre/annum. The weight of soil voided below the surface also varies considerably, from 2 to 21 tons/annum. The table also shows that a low wormcast production does not necessarily indicate a low level of activity of earthworms. This is particularly well shown on Pastures and Claycroft which have a low production of wormcasts but a high total consumption of soil due to the presence of a large population of non-casting species.

The time required for the top 4 in. of soil to pass through the alimentary tracts of the earthworm population can be calculated for four fields for which estimations of the weight of fine soil per acre to a depth of 4 in. are available. The time varies from 11½ years in old permanent pasture with a high earthworm population to 80 years for an arable field with a low earthworm population.

TABLE 7. A. *Time required for the soil in the top 4 in. to pass through the alimentary tracts of the earthworm population*

	Parklands	Pastures	Great Field III	Delharding
Years	11½	20	21	80

B. *Thickness of layer of soil brought to the surface by earthworms per annum*

Inches	0·24	0·02	0·10	0·03

Table 7 B also records the thickness of a layer of soil equivalent to the weight of wormcast production per annum. This was calculated from the apparent specific gravity of the top 4-in. layer of the field in question. The layer varies from 0·24 in. for old pasture to 0·02 in. for a 7-year-old pasture. Darwin (1881) gives estimates of the thickness of the soil layer, brought about by wormcast production, of 1–1½ in./10 years which are considerably less than values of 1·9–2·2 in./10 years deduced from the depth of burial of objects such as stones, cinders, etc., after a period of 10–30 years. The figure obtained on Parklands for the equivalent layer of soil does

approximate to that deduced by Darwin from the depth of burial. Stockli (1928) gives records of the equivalent layer of soil for several habitats. They range from 0·12 to 0·28 in. for permanent grassland down to 0·02 in. for garden soil, a range very similar to that obtained in the present investigation.

Muller (1889) carried out extensive investigations on beechwoods in Denmark and made numerous observations on the effects of earthworms on the soil. He observed that where earthworms were numerous the forest litter was incorporated in the top layers of the soil producing a good mellow soil, known as 'Mull'. Where earthworms were absent, the forest litter lay on the surface of the soil producing a layer of partially decomposed organic matter, known as 'Torf'. In the areas where earthworms were plentiful, Muller was puzzled by the presence of stones in the top 2 in. since this observation ran counter to those of Darwin. In some of the areas, the dominant earthworm was *Lumbricus castaneus* Sav., a small worm about 5 cm. in length, living in the surface layer of decaying organic matter. Muller realized that such a mode of life would clearly not result in the sinking of stones to any appreciable depths. In other areas, the dominant earthworm was the large *L. terrestris* which burrows deeply into the soil and whose activity, Muller thought, should result in a layer of stone-free soil of appreciable thickness. This apparent contradiction between the observations of Darwin and Muller is clearly due to differences in the dominant species present on the areas studied. On the pastures studied by Darwin casting species are evidently plentiful while in some areas of the beechwoods *L. terrestris*, a non-casting species, was dominant.

The effect of earthworms on soil structure

The depth of the stone-free layer on the six pasture fields under investigation was determined roughly by digging. Only two of the fields, Parklands and Great Field III, had a stone-free layer of any appreciable depth ($3\frac{1}{2}$–5 in.), the other four had stones in the surface layer and also large stones still lying on the surface. In these four fields, wormcast production since the last ploughing was sufficient only to bring about the incorporation of small and medium sized stones into the surface layer. In the two arable fields, stones of all sizes were found on the surface.

In order to gain a more accurate picture of the effect of earthworms on the structure of the soil, volume-weight determinations were carried out on three pasture fields to a depth of 8 in., and on the two arable fields to a depth of 4 in. The two samplers used were 2 in. deep and 3 and 4 in. diameter respectively. The larger size was used for the stony layer. Six sets of samples were taken in each field. The samples were air-dried before weighing and the weight of stones retained on a 2 mm. sieve was also determined.

Table 8 shows the results obtained calculated on a tons per acre basis.

In each of the three fields examined the weight of soil per acre to a depth of 8 in. is over 1000 tons/acre. The acre weights of the three fields are highly significantly different. In the two old-established fields, Parklands and Great Field III, the

weight of soil per 2 in. layer increases steadily with depth but this is not so in Pastures which has only been down to grass for 7 years. In this field the weight of soil per 2 in. layer is fairly uniform down to a depth of 8 in. and is significantly higher in the first two layers than those of Parklands and Great Field III. The weight of stones per 2 in. layer has a somewhat similar distribution except that the gradient from the surface to 8 in. is very much steeper. Very few small stones are found in the upper 2 in. layer of Parklands and Great Field III but their number and size increases rapidly with depth (Table 9). In Pastures the weight of stones per 2 in. layer is uniform down to 6 in. but the fourth layer contains a heavier weight. The total weight of stones per acre is similar in the three fields.

TABLE 8. *Weight in tons per acre of 2 in. layers of soil*

Depth in in.	Parklands	Great Field III	Pastures
1 and 2	170	198	265
3 and 4	243	237	294
5 and 6	285	274	279
7 and 8	312	370	323
Total	1010	1079	1161
Standard error of difference between 2 in. layers			±25·6
Standard error of difference between totals			±22·5

TABLE 9. *Weight of stones in tons per acre of 2 in. layers of soil*

Depth in in.	Parklands	Great Field III	Pastures
1 and 2	1	1	46
3 and 4	5	12	53
5 and 6	65	101	52
7 and 8	116	94	80
Total	187	208	231
Standard error of difference between totals			±29·5

The stones in the second 2 in. layer on Parklands and Great Field III are present at the bottom of this layer and represent the surface of the stone pavement which is well marked in these two fields. Although Parklands has about twice as many individuals of the two wormcasting species per acre as Great Field III the depth of the stone-free layer of soil is almost exactly the same in the two fields. Seven years under grass has not brought about any appreciable difference in the distribution of stones on Pastures; the upper 4 in. of this field contain 99 tons/acre and the two arable fields, Stackyard and Delharding, contain 99 and 112 tons/acre respectively to a like depth. This is clearly due to the small production of wormcasts consequent upon the low numbers of wormcasting species present in the high earthworm population of Pastures.

PORE SPACE

The pore space of the different layers of soil varies considerably (Table 10).

The pore space of Parklands decreases steadily down to 8 in., that of Great Field III down to 6 in. and that of Pastures is constant down to 6 in. with a possible

decrease in the lowest layer. In Pastures the pore space of the total depth examined is highly significantly lower than in Parklands and Great Field III, the difference clearly residing in the top 4 in. Since the weight of worms per acre for Pastures is intermediate between that for Parklands and Great Field III (8½ cwt./acre compared with 11 and 7 cwt.), it might be expected that the pore space of the top 4 in. would be similar in all three fields owing to the burrowing activities of the worms.

TABLE 10. *Percentage pore space of successive 2 in. layers of three pasture fields*

Depth in in.	Parklands	Great Field III	Pastures
1 and 2	66	61	43
3 and 4	52	53	37
5 and 6	39	43	40
7 and 8	30	44	31
0–8	47	50	38
Standard error of difference between 2 in. layers			±4·0
Standard error of difference between fields			±1·9

Since it is not so, it could be regarded as evidence that earthworms do not have an important effect on the pore space. But here again the difference in the habits of the dominant species on the three fields must be taken into consideration. On Pastures *Lumbricus terrestris* L. and *Allolobophora caliginosa* Sav. are co-dominant, each composing 30% of the population, *A. nocturna* and *A. longa* forming only 10%. On Parklands and Great Field III, *A. nocturna* and *A. longa* are the dominant species forming 45 and 44% of the population, *A. caliginosa* and *Lumbricus terrestris* forming 34 and 10% and 23 and 15% respectively on the two fields. Thus on Pastures the dominant species of worms are of the type which void excreta below the surface of the ground and so have little or no effect on pore space; on Parklands and Great Field III the dominant species void, at least, some of their excreta on the surface, thus maintaining a higher pore space in the top 4 in. layer of soil.

Consideration of the data given in Table 11 shows that the burrowing activities of the earthworm fauna of Pastures has failed to increase the pore space during the 7 years of grass following many years of continuous arable.

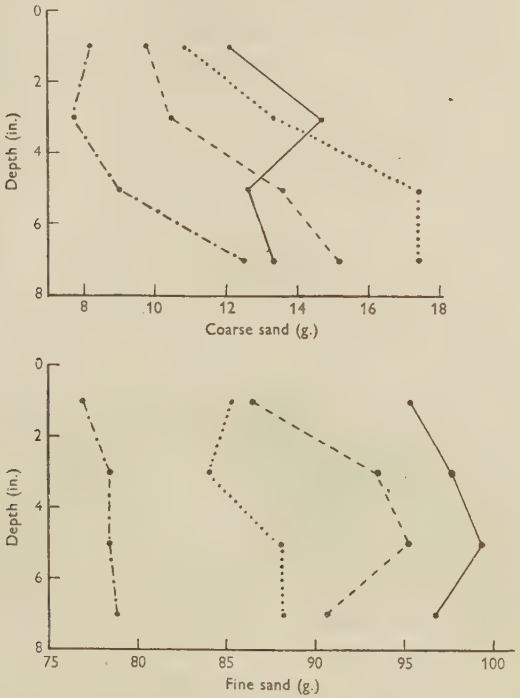
TABLE 11. *Pore space of top 4 in. layer of two arable and three pasture fields*

Arable		Grass		
Stackyard	Delharding	Pastures	Great Field III	Parklands
45	45	40	57	59
Standard error of difference		±2·1		

The pore space of the two old pastures is considerably higher than that of the arable fields but that of Pastures is significantly lower. Thus the combined action of rain and treading by stock has brought about a consolidation of the soil during the last 7 years and earthworm activity has not been able to compensate completely for this.

The effect of earthworms on the distribution of coarse and fine sand in the soil

The effect of earthworms on the distribution of stones in three pasture fields has been discussed above. Mechanical analyses were carried out to determine whether evidence could be found of any effects of earthworms on the vertical distribution of the smaller soil particles. Text-fig. 1 shows the vertical distribution of coarse and



Text-fig. 1. The distribution of coarse and fine sand in 2 in. layers on three pasture fields, expressed as g./100 g. of clay and silt. —— Pastures; ..... Great Field III; -·-·-·-·- Parklands (high population); - - - - - - Parklands (low population).

fine sand relative to the amount of silt and clay. Two curves are given for Parklands, as part of the field has a much lower earthworm population (approximately 50%) than the remainder. The vertical distribution of coarse sand on Pastures is independent of depth; on Parklands and Great Field III the proportion of coarse sand increases appreciably with depth. Particularly noticeable is the increase between the second and third 2 in. layers, that is at the depth to which the stones have sunk in the older pastures. Stockli (1928) mentions that earthworms do not swallow stones larger than 2 mm. in diameter. It seems reasonable to conclude that, in a pasture containing a high population of wormcasting species, stones of a diameter greater

than 2 mm. sink to a depth of 4–5 in. comparatively quickly, about 20 years, but that the rate of sinking of particles 0·2–2·0 mm. diameter is very much slower. The maximum size of stone particles swallowed is likely to vary according to the size of the species of earthworm concerned. Stockli's figure of 2 mm. was obtained from a mechanical analysis of wormcasts and so probably refers to large species. Thus the rejection of particles of coarse sand by small species of worms and small individuals of large species would cause these particles to sink slowly in the profile but their consumption and consequent ejection on the soil surface by large individuals of the wormcasting species would counteract this sinking to some extent. Thus the present distribution of coarse sand in the old pastures may be the result of two opposing tendencies. The distribution of fine sand in the profile is fairly uniform.

The lowest ratios of coarse sand to silt and clay occur in Parklands. Parklands is shown in the map of the Rothamsted Manor of 1623 as parkland with a number of trees. This field has been under grass for at least three centuries but Great Field III and Pastures have been ploughed up from time to time so that the activity of the earthworms on these two fields has been less compared with that on Parklands. Several workers have suggested that earthworms bring about a comminution of the soil due to the continual passage of the soil through their alimentary tracts but only two have carried out observations on the particle size distribution before and after the action of earthworms. Bassalik (1913) showed that earthworms could bring about a measurable comminution of granite particles 0·42–1·82 mm. in diameter in 3 months. Blank & Giesecke (1924) carried out a series of observations on the effects of earthworms on the physical and biological properties of soils. They kept earthworms (probably *L. terrestris*) for 2 years in pots containing different kinds of soil. At the end of this period numerous analyses were carried out including the Atterberg mechanical analysis. Their results show that when earthworms were confined in loam there was an increase in the fraction less than 0·002 mm. and a decrease in the fractions 0·02–0·06 and 0·06–0·20 mm.; in the case of loam to which sand had been added, there was an increase in the fractions less than 0·002 and 0·002–0·006 mm. accompanied by a decrease in the same two fractions as for loam alone. It is possible therefore that the lower ratios of coarse sand to silt and clay found in Parklands as compared with those in Great Field III and Pastures may be partly due to an actual reduction in the amount of coarse sand present as well as to a redistribution in the profile.

The three fields discussed above belong to the same soil type, a silty medium loam of the Batcombe series.

I am indebted to Dr E. W. Russell of the Physics Department for his advice and help in the mechanical analyses of soil, and to Mr Robin Levy for his assistance in field and laboratory work.

REFERENCES

Bassalik, K. (1913). Über Silikatzersitzung durch Bodenbacterien. *Z. Gärungsphysiol.* **11**, 1–32.

Blank, E. & Giesecke, F. (1924). Ueber den Einfluss der Regenwürmer auf die physikalischen und biologischen Eigenschaften des Bodens. *Z. Pflanz. Düngen*, B, **3**, 198–210.

Darwin, C. (1881). *The formation of vegetable mould through the action of worms*, pp. 1–326. London.

Evans, A. C. & Guild, W. J. McL. (1947). Studies on the relationships between earthworms and soil fertility. I. Biological studies in the field. *Ann. appl. Biol.* **34**, 307–30.

Muller, P. E. (1889). Récherches sur les formes naturelles de l'humus. *Ann. Sci. agron. France et étrangère, Nancy*, **6**, 85–423.

Snedecor, G. W. (1946). *Statistical methods* (fourth edition). Ames, Iowa: Collegiate Press.

Stockli, A. (1928). Studien über den Einfluss des Regenwürmer auf die Beschaffenheit des Bodens. *Landw. Jb Schweiz.* **42**, 1–121.

(*Received 2 July* 1947)

SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

IX. INTERACTION BETWEEN HOST PLANT NUTRITION, DISEASE ESCAPE, AND DISEASE RESISTANCE

By S. D. GARRETT

Rothamsted Experimental Station, Harpenden, Herts

In a field experiment, the disease rating of the roots of barley plants suffering from 'take-all' was reduced by application of nitrogen, and by a combined dressing of phosphate and potash. This reduction is attributed to the fact that manuring enables the cereal plant to produce new crown roots more quickly than *Ophiobolus graminis* can infect them. In an earlier pot-culture experiment, operation of this disease-escape mechanism was inadvertently reduced by inoculating plants at the crown, and by environmental conditions exceptionally favourable to infection (sand culture and relatively high temperatures in the glasshouse). Under these conditions, not only was root disease rating as high in the series receiving a full supply of nutrients (NPK) as in that receiving only one-third the full amount, but it was reduced almost to one-half in the series receiving one-third nitrogen in the presence of full phosphate and potash ($PK\frac{1}{3}N$). It is concluded that an increase in nitrogen supply may increase the intrinsic susceptibility of individual roots to infection, at the same time as it promotes disease escape and increases yield of the whole plant.

During recent years, the beneficial effect of artificial fertilizers in reducing the incidence of 'take-all' (caused by *Ophiobolus graminis* Sacc.) has received especial attention, not only in this country but also in Australia and the U.S.A. (Garrett, 1946). Further evidence of this effect has been obtained from a field experiment in progress during the past 2 years at the Woburn Experimental Station. It is proposed to present here some interim data from this experiment, in order to demonstrate and resolve an apparent contradiction that has arisen between these field results, and some other results obtained several years ago in a nutrient sand culture experiment in glasshouse pots (Garrett, 1941).

Autumn-sown wheat occupied the experimental site (approximately 1 acre) in 1944, and was followed by spring-sown Plumage Archer barley in 1945 and again in 1946. Natural inoculum of *O. graminis* was present throughout the soil of the experimental area; in sufficient abundance to give a satisfactory development of 'take-all' in the barley crops of 1945 and 1946. The experiment, of factorial design, comprised forty-eight plots of 1/50 acre each. Twelve plots were allotted to each of four different fertilizer treatments, all applied a few weeks after sowing* as follows: (1) no fertilizers; (2) 0·4 cwt./acre P_2O_5, as superphosphate, +0·5 cwt./acre K_2O, as muriate of potash; (3) 0·4 cwt./acre N, as sulphate of ammonia; (4) 0·4 cwt. N + 0·4 cwt. P_2O_5 + 0·5 cwt. K_2O/acre. It is not proposed to discuss here the effects of the other treatments incorporated in this experiment, and so no particulars of

* With the single exception that the wheat received sulphate of ammonia as a top-dressing in April, 7 months after sowing.

these other treatments will be given. The fertilizer treatments given to the 1944 wheat crop were applied to the same plots in 1945 and again in 1946, so that in 1946 a cumulative effect of fertilizer treatment upon incidence of 'take-all' was obtained.

Estimation of disease. In 1945, ten 8 in. samples of drill row were taken at random from each of the forty-eight plots just after harvest, on 15 August. In 1946, twelve 24 in. samples of drill row were taken at random from each plot whilst the crop was still standing, on 9 July. The samples were at once dried and stored for subsequent examination. After the roots had been washed free of soil, samples were separated into individual plants, which were classified by inspection into one of the following three categories:

Class I. Infection light or none 0 marks.
Class II. Infection medium 1 mark.
Class III. Infection severe 2 marks.

The percentage disease rating for each individual sample was calculated as follows:

$$\frac{\text{Sum of numerical ratings of individual plants}}{\text{Number of plants} \times 2} \times 100.$$

The percentage disease rating for each plot was then calculated by taking the mean of the disease ratings for the ten or twelve individual samples.

Effect of fertilizer treatments on disease rating

The mean disease ratings for the four groups of twelve plots receiving the different fertilizer treatments are given in Table 1.

Table 1. *Percentage disease ratings*

	No fertilizers	PK only	N only	NPK
In 1945	39	35	23	16
In 1946	39	33	27	22

The effect of nitrogen in reducing D.R.* is highly significant ($P < 0{\cdot}001$), both in 1945 and in 1946. The effect of phosphate + potash in reducing D.R. is significant ($P < 0{\cdot}01$) in 1946, but not in 1945.

These results are apparently at variance with those reproduced in Table 2, previously reported by Garrett (1941), working with plants grown in pots of sand + nutrient solution.

Table 2. *Effect of fertilizers on root-disease rating and yield of plants grown in sand culture (reproduced from Garrett, 1941)*

	Mean number ear-bearing tillers per plant	Percentage tillers with majority of roots severely infected	Percentage reduction in yield due to infection
NPK	5·5	66	3
PK$\frac{1}{3}$N	4·3	35	24
NK$\frac{1}{3}$P	4·9	68	49
NP$\frac{1}{3}$K	4·8	79	21
$\frac{1}{3}$(NPK)	4·3	65	43

* D.R. = disease rating.

In this experiment, D.R. was as high in the series receiving a full supply of nutrients (NPK) as in that receiving only one-third the full amount ($\frac{1}{3}$(NPK)). Moreover, in series PK$\frac{1}{3}$N, D.R. was reduced nearly to one-half. In this pot experiment, therefore, a deficiency of N in the presence of adequate PK actually reduced D.R., whereas in the field experiment a deficiency of N consistently increased D.R., both in the presence and absence of PK dressings.

The clue to this anomaly is perhaps to be found in the discrepancy between the method of inoculation employed in the pot-culture experiment, and natural infection as it occurs in the field. In the field, inoculum of *O. graminis* is usually so dispersed throughout the soil that the majority of the first-formed roots must grow for some distance from the crown of the plant before they come into contact with the fungus. It is comparatively rare for seedling cereal plants to come early into heavy contact with the fungus and die in the seedling stage (the 'take-all' form of this disease); the majority of plants become infected more gradually, and die after heading (the 'whiteheads' form of the disease). Now it is evident that production of new crown roots by the cereal plant must be greatly increased by adequate manuring with NPK. Until *O. graminis* has established infection of the crown itself, many of the new crown roots thus produced may escape infection for a considerable period. The chances of new crown roots for *disease escape* dwindle with the approach of *O. graminis* to the crown of the plant; the speed of its approach depends upon (i) the original concentration and distribution of inoculum in the soil, (ii) the growth rate of the runner hyphae along the roots, which varies with soil conditions (Garrett, 1936; Winter, 1939, 1940). Granted a distribution of inoculum which is not unusually heavy, and a soil which is not exceptionally favourable to the spread of the runner hyphae, the application of NPK must lower D.R., merely by increasing the gross production of new crown roots at a rate greater than the fungus can cause them to become visibly diseased (i.e. because D.R. is based upon the ratio between apparently healthy (white) and obviously diseased (discoloured) crown roots). With inadequate supplies of NPK, on the other hand, production of new crown roots may be so reduced that D.R. increases steadily with the approach of maturity, until all roots are obviously diseased.

In the pot-culture experiment, an attempt was made to imitate conditions of infection obtaining in the field, by placing a layer of inoculum at a distance of 10 cm. below seed level. Unfortunately, insufficient allowance was made for drainage from the upper levels of sand in these pots, with the result that a water table developed, and no infection occurred from the inoculum buried 10 cm. deep. A second inoculation was therefore made 1 month after sowing, with two pieces of infected straw, placed on either side of and immediately below the crown of each plant. This placing of the inoculum, together with the exceptionally favourable conditions offered by pure sand for spread and infection by *O. graminis* (Garrett, 1936), must greatly have reduced chances of disease escape for new crown roots. Moreover, soil temperatures were considerably higher in the glasshouse pots than in the field, and

this must further have favoured the fungus at the expense of the host plant; the optimum temperature for development of take-all lies well above 20° C. (Henry, 1932; Garrett, 1934, and 1942, p. 31). Each of these three factors probably contributed towards the final result, viz. that D.R. for roots was as high in the series with full NPK manuring as in that receiving only one-third NPK (Table 2). A further point of particular interest in Table 2 is the reduction of D.R., almost to half, in the series PK$\frac{1}{3}$N. It seems that here, *under conditions tending to reduce the disease escape mechanism to a minimum, an effect of nutrient ratio upon the intrinsic susceptibility to infection of root tissues can be discerned.* The figures in Table 2 indicate that intrinsic susceptibility of root tissues is not affected by a deficiency of all three nutrients together, but is decreased only by a deficiency of N in the presence of full P and K. Conversely, D.R. is higher for series NP$\frac{1}{3}$K, with an unbalanced deficiency of K, than for any other series.

It is difficult to avoid the conclusion, therefore, that field resistance of the cereal plant to take-all is conditioned by at least two factors: (1) a disease-escape mechanism inherent in the capacity of the plant to produce a succession of new crown roots (Simmonds & Sallans, 1933); (2) the intrinsic susceptibility of the tissues of individual roots to infection by the fungus. Under some circumstances at least, the application of nitrogen, unbalanced by equivalent additions of phosphate and potash, may actually increase the visible degree of infection in some individual roots, at the same time as it increases crown-root production and grain yield, and lowers D.R. for the plant as a whole.

Grateful acknowledgement is made to Dr H. H. Mann for conducting the field experiment, from which these data have been taken, at the Woburn Experimental Station.

REFERENCES

Garrett, S. D. (1934). Factors affecting the severity of take-all. II. Soil temperature. *J. Dep. Agric. S. Aust.* **37**, 799.

Garrett, S. D. (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

Garrett, S. D. (1941). Soil conditions and the take-all disease of wheat. VI. The effect of plant nutrition upon disease resistance. *Ann. appl. Biol.* **28**, 14.

Garrett, S. D. (1942). The take-all disease of cereals. *Tech. Commun. Bur. Soil Sci., Harpenden*, **41**.

Garrett, S. D. (1946). Reduction of take-all by artificial fertilizers. *J. Minist. Agric.* **53**, 223.

Henry, A. W. (1932). The influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis*. *Canad. J. Res.* **7**, 198.

Simmonds, P. M. & Sallans, B. J. (1933). Further studies on amputations of wheat roots in relation to diseases of the root system. *Sci. Agric.* **13**, 439.

Winter, A. G. (1939). Der Einfluss der physikalischen Bodenstruktur auf den Infektionsverlauf bei der Ophiobolose des Weizens. *Z. PflKrankh.* **49**, 513.

Winter, A. G. (1940). Weitere Untersuchungen über den Einfluss der Bodenstruktur auf die Infektion des Weizens durch *Ophiobolus graminis*. *Zbl. Bakt.* Abt. 2, **101**, 364.

(*Received* 14 *June* 1947)

THE ROLE OF ANTIBIOTICS IN THE DECOMPOSITION OF SAWDUST

II. INHIBITION OF THE GROWTH OF CELLULOSE-DECOMPOSING FUNGI

By S. E. JACOBS and A. W. MARSDEN
Imperial College of Science and Technology, London

The action of the antibiotics present in deal sawdust on the growth on Czapek-Dox agar of cellulose-decomposing fungi has been examined. *Stachybotrys atra* and *Chaetomium indicum* were strongly inhibited by substances in cold-water extracts, but *C. globosum* only slightly so. The extracts also contained material which stimulated the growth of *C. globosum* but not that of the other two fungi. The formation of perithecia by *C. indicum* and *C. globosum* was also stimulated by the extract. There was no marked inhibition or stimulation of the growth of *Aspergillus terreus*, *A. fumigatus*, or three species of *Penicillium* by the extracts.

In an earlier communication (Jacobs & Marsden, 1947) it was shown that deal sawdust contained substances capable of inhibiting the growth of cellulose-decomposing bacteria of the genera *Sporocytophaga* and *Cellulomonas*. In addition, the sawdust was strongly acid, and this alone would have sufficed to produce inhibition. Sawdust could therefore only be expected to be attacked by these bacteria if the acidity were first neutralized. When this had been done the antibiotics present still completely inhibited the growth of *Sporocytophaga myxococcoides* on filter-paper in the presence of sawdust, and delayed the growth of a species of the genus *Cellulomonas*. For this reason alone these particular species are unlikely to be of great use for the decomposition of sawdust, although the cellulose in wood-dust has been successfully fermented by both thermophilic and mesophilic bacteria (Virtanen, 1946). The finer the wood was ground the greater was the extent of the decomposition.

Many species of fungi capable of decomposing cellulose are known. These are able to grow in acid conditions, so that prior neutralization of the sawdust would not be required. Nevertheless, neutralization might on general grounds be desirable, both of a decomposed product designed to be added to the soil, and of sawdust added to soil to be decomposed therein. Fungi are known to be largely concerned in the decomposition of cellulose in soils (Waksman & Skinner, 1926), and as a result of their activities they contribute to the soil humus (Jensen, 1931). It was therefore considered that an investigation of the action of the sawdust antibiotics on cellulose-decomposing fungi would prove profitable since, other things being equal, species resistant to these substances would be more valuable for the decomposition of sawdust. However, for the organism to be completely satisfactory the property of resistance would need to be combined with the ability to overcome the barrier offered by the lignin present.

Experimental

(*a*) *Materials.* As in the experiments with bacteria previously reported (Jacobs & Marsden, 1947), deal sawdust derived from a variety of white coniferous woods was used. This consisted entirely of clean dry particles, and only the portion passing a 2 mm. sieve was employed.

The fungi tested comprised eight species, all of which were known to be able to decompose the cellulose of filter-paper. All except one of these organisms were isolated by Dr S. N. Basu of the Indian Jute Mills Association Research Institute, and the authors are greatly indebted to him for supplying the cultures. They were obtained (with the exception of *Stachybotrys atra*, which came from cotton) from spoiled jute fibre, and were considered to be especially well suited for use in the experiments to be described, since jute is highly lignified and these fungi might therefore also be able to attack sawdust, another lignified material. The species used were: *Chaetomium globosum*, *C. indicum*, *Stachybotrys atra*, *Aspergillus terreus*, *A. fumigatus*, and three strains of *Penicillium*. The latter were all members of the group of Biverticillata-Symmetrica, one being a strain of *P. funiculosum*. Another of the *Penicillium* species produced perithecia when first isolated but has now lost this property.

(*b*) *Methods.* The presence of antifungal substances in the sawdust was demonstrated by growing the fungi on solid Czapek-Dox medium in which had been incorporated various amounts of an aqueous extract of sawdust, and comparing the diameters of the fungal colonies on the extract-containing media with those on the control medium.

A 1 in 10 (wt./vol.) suspension of sawdust in distilled water was made, allowed to stand at room temperature for 4 days, and then filtered. In this extract the mineral salts of the Czapek-Dox medium were dissolved, and as the aqueous extract had a *p*H of 5·2 the monobasic potassium phosphate was used instead of the dibasic salt, in order that the final medium should have approximately the same reaction. When the ferrous sulphate constituent was added a dull purple colour developed, indicating that phenolic substances were present. A solution of the mineral salts of the medium in distilled water was also made, and added to the extract medium to give others containing 50, 10 and 1% of the extract. Portions of these four media, and also the control, were then provided with 3% of sucrose and 2% of agar, the latter being dissolved and the media simultaneously sterilized by autoclaving at 120° C. for 20 min. Plates were then poured, each 8·5 cm. plate receiving approximately 15 ml. of medium. When set, the plates were inoculated in the centre, using the tip of a straight wire carrying spores from a slant culture. These plates were incubated at 30° C. and at intervals the colony diameters were measured to the nearest mm. The figures given in the tables are the means of two measurements taken at random, this number being considered sufficient as the colonies were always nearly circular.

(*c*) *Results of inhibition experiments*. The results of a preliminary experiment on three fungi, *Chaetomium globosum*, *C. indicum* and *Stachybotrys atra*, are given in Table 1. The colonies were only measured once, after 3 days' incubation, but it is evident that inhibitory substances were present, although the three fungi reacted differently. *S. atra* was strongly inhibited by 100% of extract, less so by 50%, while lower concentrations were ineffective. *Chaetomium indicum* was inhibited markedly by 100 and 50% of extract and there may have been a slight retardation of growth even at 1%. *C. globosum*, however, was not inhibited even by 100% of extract, and with smaller amounts there appeared to have been a slight stimulation. A further point of difference between the behaviour of *C. globosum* and *C. indicum* was in the production of perithecia. The former had produced large perithecia easily visible to the naked eye in the presence of 100% of extract, but at lower concentrations and in the control they were not visible. The latter had formed visible perithecia on all the extract media, though not in the control.

TABLE 1. *The effect of different concentrations of aqueous sawdust extract on the growth of* Chaetomium globosum, C. indicum *and* Stachybotrys atra

Concentration of extract (%)	Mean diameter of colonies in mm.*		
	C. globosum	*C. indicum*	*S. atra*
100	25†	17†	4
50	30	26†	11·5
10	30	35†	17
1	30	35†	15
Control: no extract	25	40	15

* On Czapek-Dox agar at *p*H 5·0 after incubation for 3 days at 30° C.
† Perithecia visible to the naked eye.

From the observations recorded above it appeared likely that the sawdust extract may have contained both inhibitory and stimulatory substances. These could in certain circumstances neutralize each other, producing no apparent effect. It was therefore decided to repeat the experiment, and to measure the colony diameter at intervals during several days' incubation. The results, presented in Table 2, show that *Stachybotrys atra* was inhibited markedly by 100% extract, the effect being still visible on the ninth day. 50% of extract caused an inhibition which was overcome by the sixth day. This suggests that an adaptation by the fungus had occurred. As before, 10% of extract was without effect. *Chaetomium indicum* showed inhibition by 100 and 50% of extract, although the effect of the latter concentration was relatively slight, but there was no suggestion of any growth stimulation with this species, or with *Stachybotrys atra*. *Chaetomium globosum*, however, showed very marked stimulation of growth in 100 and 50% of extract after 6 days' incubation, although 10% was without effect. Also, with 100% of extract there was a definite inhibition of growth for 2 days, after which the fungus apparently adapted itself and

became able to take advantage of the stimulatory material. This effect was missed in the previous experiment because the observations were made only once, after 3 days' growth and, as will be seen from Table 2, at a time when the initial inhibition had just disappeared. As before, the speed of development of perithecia to the stage at which they were visible to the naked eye was greatly enhanced in *C. globosum* by a high concentration of extract. *C. indicum*, on the other hand, was stimulated at all the concentrations tested. This experiment has been repeated with substantially the

TABLE 2. *The effect of different concentrations of aqueous sawdust extract on the development of colonies of* Chaetomium globosum, C. indicum *and* Stachybotrys atra

	Mean diameter of fungal colonies in mm.*														
Concentration of extract	*C. globosum* (days)					*C. indicum* (days)					*S. atra* (days)				
(%)	1	2	3	6	9	1	2	3	6	9	1	2	3	6	9
100	2	9	23	53†	72†	1	10	21	48†	68†	0	1	2·5	15	24
50	4	15	28·5	44	49	4	14	27·5	56†	74†	1	3·5	8	21·5	29
10	5	17	25	27	31	3	15	29	63†	85†	1·5	6	11·5	24	33
Control: no extract	4	16	24	28	33	4	18	31·5	65	80	1	6	12	22	32

* On Czapek-Dox agar of *p*H 5·0 at 30° C. † Perithecia visible to the naked eye.

same results, although it appears that an extract made by treating sawdust for 3 days at 25° C. produces stronger inhibitory effects on all three organisms.

Experiments were also made with two species of *Aspergillus* and three of *Penicillium*. The results obtained with *Aspergillus terreus* and *A. fumigatus* are given in Table 3 and those with the three *Penicillium* species in Table 4, and it is clear that

TABLE 3. *The effect of sawdust extract on the growth of* Aspergillus terreus *and* A. fumigatus

	Mean diameter of colonies in mm.*								
Concentration of extract	*A. terreus* (days)					*A. fumigatus* (days)			
(%)	1	2	3	6	9	1	2	3	6
100	2	13	23	58	80	2	18	37·5	85†
50	2	13	22	53	78	2	22	40	85
10	2·5	13	21	46	73	2	25	42	85
Control: no extract	2	13	22	53	73	2·5	23	40	85

* On Czapek-Dox agar of *p*H 5·0 at 30° C. † The whole of the dish was covered.

there was no definite inhibition or stimulation at any stage with any of these five fungi. Repetitions of these experiments gave similar results, but very slight inhibitions in the early stages of growth were produced by 100% of the stronger extract referred to above.

TABLE 4. *The effect of sawdust extract on the growth of three species of* Penicillium

Concentration of extract (%)	Mean diameter of colonies in mm.*														
	P. funiculosum (days)					*Penicillium* 40 (days)					*Penicillium* 77 (days)				
	1	2	3	6	9	1	2	3	6	9	1	2	3	6	9
100	1·5	8·5	14	32	42	1·5	7·5	10	—	—	0	3	5	15	22
50	2	10	16·5	36	52	2·5	8·5	12	23	30	0	3	5	13	—
10	2	9	16	33	49	4·5	10	12·5	23	32	0	1	3·5	14	21
Control: no extract	1	8	13·5	30	45	2·5	9	12·5	23	33	1	3·5	5	10·5	16

* On Czapek-Dox agar of *p*H 5·0 at 30° C.

DISCUSSION

The experiments reported above have shown that certain fungi of the genera *Chaetomium* and *Stachybotrys* are markedly inhibited by substances which can be extracted from sawdust with cold water, while others, of the genera *Aspergillus* and *Penicillium*, proved indifferent to them. The fact that a dull purple colour developed when ferrous sulphate was added to the extract supports the suggestion that the active substances were of a phenolic nature. Since ferric chloride is known to act as a detoxifying agent against phenols (Flett, Haring, Guiteras & Shapiro, 1945) it is possible that the iron in the medium had reduced the activity somewhat. No attempt was made to improve the potency of the extracts, and it is possible that had this been done, e.g. by the use of mild alkali, which was shown previously (Jacobs & Marsden, 1947) to extract a greater proportion of the antibacterial substances from sawdust, some inhibition would have been obtained even with the *Aspergillus* and *Penicillium* species. Nevertheless, it would be legitimate to infer that these organisms would be more likely to prove successful in decomposing sawdust cellulose at a useful rate than species such as *Chaetomium indicum* or *Stachybotrys atra* which were inhibited to a considerable degree. The case is otherwise with the species *Chaetomium globosum*. Here, although there was some initial inhibition by a high concentration of the extract, this was soon overcome and the fungus actually made better growth after a week in the presence than in the absence of the extract. This fungus apparently readily acquires resistance to the sawdust antibiotics and also is able to utilize the water-soluble material present. Because of this latter ability there might at first be a sparing action on the cellulose of the sawdust, but if the water-soluble material were not too abundant the ultimate result might be a greater cellulose decomposition because of the stimulation given to the formation of mycelium. Here it may be recalled that in the experiments with bacteria previously reported (Jacobs & Marsden, 1947) there appeared in the end to be a more vigorous destruction of cellulose in the presence than in the absence of sawdust extract, despite an initial inhibition of growth. *C. globosum*, although slightly sensitive to the sawdust antibiotics, would seem to merit a trial as an agent for the decomposition of this material.

Melin & Wickén (1946) refer to the presence of substances with anti-fungal activity in cold-water extracts of the pure forest litter of Swedish trees. The types of fungi inhabiting and decomposing the litter were resistant, but after the extracts had been autoclaved their activity was increased and the litter-inhabiting fungi were then inhibited. In the work described above, the cold-water sawdust extracts were in all cases autoclaved before being tested, and although it is not known whether the activity towards *Chaetomium* and *Stachybotrys* was thereby increased, it is clear that some species of cellulose-decomposing fungi were virtually unaffected by the autoclaved extracts. Melin & Wickén (1946) showed as well that extracts from the litter of *Acer platanoides* L. contained substances active against *Staph. aureus*, and also others antagonistic to the action of the growth inhibitors, and that the latter were destroyed by heat. Substances with a similar antagonistic action may have been present in the sawdust extracts, but if so, and they had in fact been responsible for the failure to obtain inhibition of the *Aspergillus* and *Penicillium* species tested, then they must presumably have been stable to heat. Actually, the stimulatory action observed concerned *Chaetomium globosum* alone of the fungi tested, and as in the end the growth was better in the presence than in the absence of the extract it is preferred to regard this effect as a response to an improvement in the nutrition of the fungus, although it is not impossible that specific antagonists may also have been present.

Another sign of the stimulation of *C. globosum* was the accelerated formation of perithecia in the presence of large amounts of sawdust extract, an effect also shown by *C. indicum* at lower concentrations. Basu (1946) has observed a similar stimulation of this strain of *C. globosum* with aqueous extracts of jute, and is engaged on an investigation of the factors involved. Hawker (1936) showed that the fungus *Melanospora destruens* and other ascomycetes were stimulated to produce fruiting bodies by the addition to the medium of a small quantity of an extract of lentils, and later was able to identify the active material with vitamin B_1 (Hawker, 1939). Other workers (Leonian & Lilley, 1937; Nickerson & Thimann, 1941, 1943) have also described the stimulation of sexually formed spores by growth factors, and it may well be that the behaviour of *Chaetomium globosum* and *C. indicum* reported above is but another example of this.

REFERENCES

Basu, S. N. (1946). Personal communication.

Flett, L. H., Haring, R. C., Guiteras, A. F. & Shapiro, R. L. (1945). The revival of organisms presumably killed by phenol. *J. Bact.* **50**, 591–5.

Hawker, L. E. (1936). The effect of certain accessory growth substances on the sporulation of *Melanospora destruens* and of some other fungi. *Ann. Bot.* **50**, 699–718.

Hawker, L. E. (1939). The nature of the accessory growth factors influencing growth and fruiting of *Melanospora destruens* Shear, and of some other fungi. *Ann. Bot.* (n.s.), **3**, 657–76.

JACOBS, S. E. & MARSDEN, A. W. (1947). The role of antibiotics in the decomposition of sawdust. I. Inhibition of the growth of cellulose-decomposing bacteria. *Ann. appl. Biol.* **34**, 276–85.

JENSEN, H. L. (1931). The microbiology of farmyard manure decomposition in soil. II. Decomposition of cellulose. *J. agric. Sci.* **21**, 81–100.

LEONIAN, L. H. & LILLEY, V. G. (1937). Partial purification of a vitamin-like substance which stimulates sexual reproduction in certain fungi. *Amer. J. Bot.* **24**, 700–2.

MELIN, E. & WICKÉN, T. (1946). Antibacterial substances in water extracts of pure forest litter. *Nature, Lond.*, **158**, 200–1.

NICKERSON, W. J. & THIMANN, K. V. (1941). The chemical control of conjugation in *Zygosaccharomyces*. *Amer. J. Bot.* **28**, 617–21.

NICKERSON, W. J. & THIMANN, K. V. (1943). The chemical control of conjugation in *Zygosaccharomyces*. II. *Amer. J. Bot.* **30**, 94–101.

VIRTANEN, A. I. (1946). Fermentation of wood-dust by cellulose bacteria. *Nature, Lond.*, **158**, 795.

WAKSMAN, S. A. & SKINNER, C. E. (1926). Micro-organisms concerned in the decomposition of celluloses in the soil. *J. Bact.* **12**, 57–84.

(*Received* 3 *June* 1947)

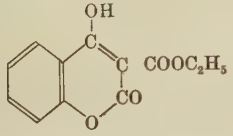
THE FUNGISTATIC ACTIVITY OF ETHYLENIC AND ACETYLENIC COMPOUNDS

I. THE EFFECT OF THE AFFINITY OF THE SUBSTITUENTS FOR ELECTRONS UPON THE BIOLOGICAL ACTIVITY OF ETHYLENIC COMPOUNDS

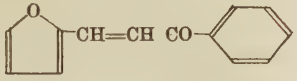
By J. C. McGOWAN, P. W. BRIAN and H. G. HEMMING
Imperial Chemical Industries Limited Butterwick Research Laboratories, The Frythe, Welwyn, Herts

Over eighty compounds have been assayed for fungistatic activity. *Fusarium graminearum*, *Penicillium digitatum* and *Botrytis allii* were used as test organisms. The preliminary results suggest that fungistatic activity is associated with the tendency of the substituents to withdraw electrons from the double bond. Nitroethylenes and fumarates are, for example, fungistatic. Tetraiodoethylene has high activity but this can hardly be attributed solely to the withdrawal of electrons from the double bond by the iodine atoms.

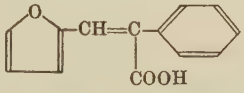
In the course of routine evaluation of a large number of organic compounds for fungistatic activity it was observed that amongst the most promising compounds were a number containing only carbon, hydrogen and oxygen. Examples of such substances are the ethyl ester of 4-hydroxycoumarin-carboxylic acid-3, furfural-acetophenone, and furfurylidene-phenylacetic acid.



Ethyl ester of 4-hydroxycoumarin-carboxylic acid-3



Furfuralacetophenone



Furfurylidene-phenylacetic acid

Since nearly all of these fungistatic compounds contained an ethylenic double bond a study was undertaken of the factors required to produce fungistatic activity in an ethylenic compound. It was hoped that such a study might throw some light on the activity of the powerfully fungistatic mould metabolic products viridin (Brian & McGowan, 1945; Brian, Curtis, Hemming & McGowan, 1946) and glutinosin (Brian & McGowan, 1946) both of which contain only carbon, hydrogen and oxygen.

Experimental materials and methods

The compounds were prepared and purified according to the methods described in the literature. The methacrylic acid and its esters were kindly supplied by the Research Department of I.C.I. Plastics Division.

Two methods have been used for assessment of fungistatic activity; these are described in turn below.

Botrytis allii *spore germination assay*

The substance to be evaluated is dissolved in sterile water, a 0·2% solution being prepared if the material is sufficiently soluble. In the case of water-insoluble substances a suspension is made by dissolving in a little alcohol or acetone and adding to sterile water, or by grinding up with water with a little Dispersol T. This solution or suspension is then assayed by a method which has previously been described in detail (Brian & Hemming, 1945). Briefly, this involves observations of the germination of *B. allii* spores in dilutions of the test solution made in $\times 2$ steps with a synthetic nutrient at *p*H 3·5.

Agar plate test

The substance to be evaluated is incorporated in solution or in suspension (prepared as above) in sterile 2% malt agar in Petri dishes. The concentrations of toxic substance used are 400, 80, 16 and 3·2 μg./ml. (occasionally the highest concentration tested has been 2000 μg./ml.). Two plates of each concentration and two control plates containing no toxic substance are inoculated with a suspension of spores of *Fusarium graminearum* and a similar set with spores of *Penicillium digitatum*. After incubation at 25° C. for 6 days the degree of growth is recorded on an arbitrary scale.

In Table 1 the results obtained are summarized; in each case the least concentration completely inhibiting growth or germination is recorded. This table is compiled from the result of a number of independent tests but salicylanilide was, on each occasion, included as a standard giving identical results.

Discussion

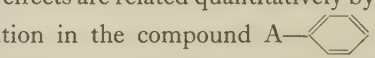
Inspection of the table shows that the greatest fungistatic activity is shown by compounds in which a strongly electron-attracting group is attached to one of the double-bonded carbon atoms. It is well known that such groups render adjacent methylene groups reactive, have acidic hydroxyl derivatives and give phenyl derivatives which are substituted in the meta position by most of the common reagents (kationoid reagents). The two latter effects are related quantitatively by the following rule (McGowan, 1936): 'Substitution in the compound A—  by kationoid reagents will give largely ortho- and para-derivatives if the dissociation constant $K = \frac{[AO^-]\,[H^+]}{[AOH]}$ of the compound AOH (measured in dilute aqueous solution at

TABLE 1. *Fungistatic activity of various ethylenic compounds*

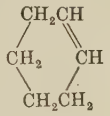
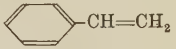
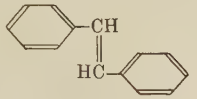
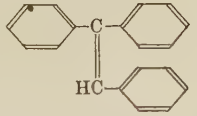
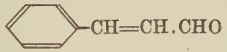
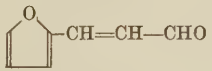
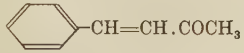
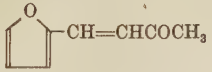
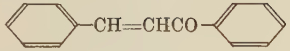
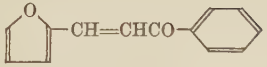
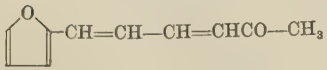
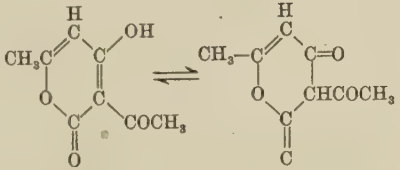
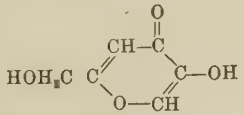
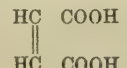
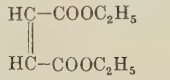
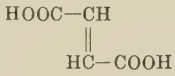
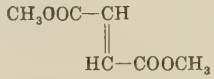
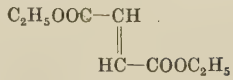
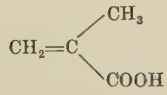
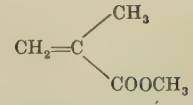
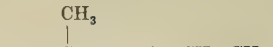
Code no.	Compound	*B. allii* spore germination test (Least conc. (μg./ml.) inhibiting germination)	Agar plate test (Least conc. (μg./ml.) inhibiting growth)	
			F. graminearum	*P. digitatum*
	HYDROCARBONS			
1.	 Cyclohexene	1000	>400	>400
2.	 Styrene	500	>400	>400
3.	 *trans*-Stilbene	>50	>400	>400
4.	 Triphenylethylene	>500	>400	>400
	ALDEHYDES AND KETONES			
5.	CH_3—CH=CH—CHO Crotonaldehyde	500	400	400
6.	 Cinnamic aldehyde	125	80	80
7.	 Furfuracrolein	1000	400	400
8.	 Benzalacetone	500	400	400
9.	 Furfuralacetone	—	400	400
10.	 Benzalacetophenone	50	>400	>400
11.	 Furfuralacetophenone	50	400	400

TABLE I (*continued*)

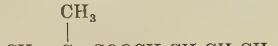
Code no.	Compound	*B. allii* spore germination test (Least conc. (μg./ml.) inhibiting germination)	Agar plate test (Least conc. (μg./ml.) inhibiting growth)	
			F. graminearum	*P. digitatum*
12.				
	1-(Furyl)-hexadiene-(1:3)-one-5	—	400	400
13.				
	1-Dimethylamino-5(furyl)-Δ⁴-penten-3-one hydrochloride	>1000	400	400
14.				
	Dehydracetic acid	50	80	400
15.				
	Kojic acid	>1000	>400	>400
	ACIDS AND ESTERS			
16.				
	Maleic acid	>1000	>400	>400
17.				
	Ethyl maleate	>1000	400	400
18.				
	Fumaric acid	>1000	>2000	2000
19.				
	Methyl fumarate	250	400	80
20.				
	Ethyl fumarate	500	80	400
21.				
	Methacrylic acid	500	400	>400
22.				
	Methyl methacrylate	>1000	>400	>400

23. $CH_2=CHCOOC_2H_5$

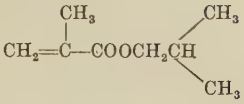
Ethyl acrylate | > 1000 | > 400 | > 400

24. 

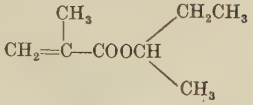
Allyl methacrylate | > 1000 | > 400 | > 400

25. 

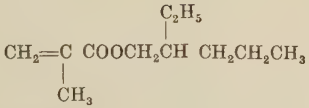
n-Butyl methacrylate | > 1000 | > 400 | > 400

26. 

Isobutyl methacrylate | > 1000 | > 400 | > 400

27. 

sec.-Butyl methacrylate | > 1000 | > 400 | > 400

28. 

2-Ethylhexyl methacrylate | > 1000 | > 400 | > 400

29. 

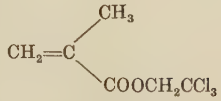
n-Amyl methacrylate | > 1000 | > 400 | > 400

30. 

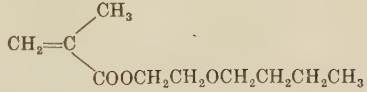
Benzyl methacrylate | 1000 | > 400 | > 400

31. 

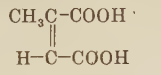
Phenylethyl methacrylate | > 1000 | > 400 | > 400

32. 

Trichlorethyl methacrylate | > 1000 | > 400 | > 400

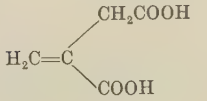
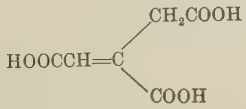
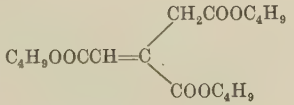
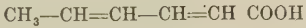
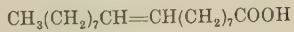
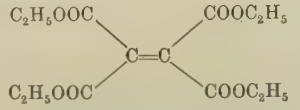
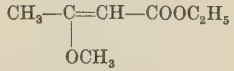
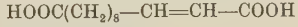
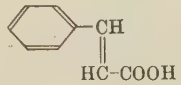
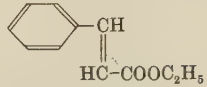
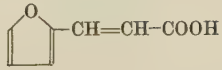
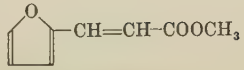
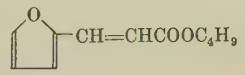
33. 

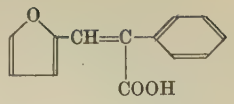
Butylcarbitol methacrylate | 1000 | > 400 | > 400

34. 

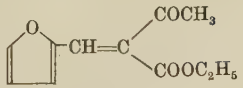
Citraconic acid | 500 | > 400 | > 400

TABLE 1 (*continued*)

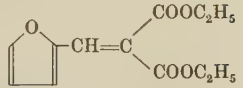
Code no.	Compound	*B. allii* spore germination test (Least conc. (μg./ml.) inhibiting germination)	Agar plate test (Least conc. (μg./ml.) inhibiting growth)	
			F. graminearum	*P. digitatum*
35.	 Itaconic acid	>1000	>2000	>2000
36.	 Aconitic acid	>1000	2000	2000
37.	 Tri-*n*-butyl aconitate	—	>400	>400
38.	 Sorbic acid	62·5	400	400
39.	 Elaidic acid	>1000	>400	>400
40.	 Tetra-ethyl ester of ethylene-tetracarboxylic acid	>50	400	>400
41.	 Methoxycrotonic ester	500	>400	>400
42.	 Traumatic acid	31·25	—	—
43.	 Cinnamic acid	250	400	400
44.	 Ethyl cinnamate	>50	400	400
45.	 Furylacrylic acid	62·5	>400	>400
46.	 Methyl furylacrylate	>50	>400	400
47.	 *n*-Butyl furylacrylate	—	400	>400

48. 

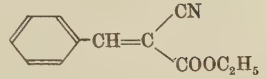
Furfurylidene phenylacetic acid — 12·5 — 400 — >400

49. 

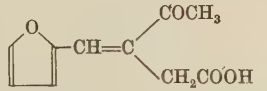
Ethyl furfurylideneacetoacetate — 250 — 400 — 400

50. 

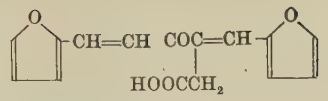
Diethyl ester of furfurylidene malonic acid — 250 — 400 — 400

51. 

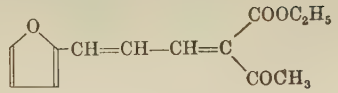
Ethyl α-cyano-β-phenylacrylate — 250 — 400 — >400

52. 

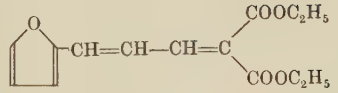
β-Furfurylidene laevulinic acid — >1000 — >400 — >400

53. 

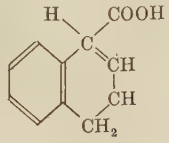
$\beta\delta$-Difurfurylidene laevulinic acid — >1000 — >400 — >400

54. 

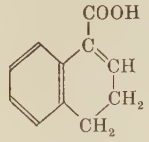
Ethyl ester of γ-furfurylidene-α-acetocrotonic acid — — — 400 — >400

55. 

Ethyl ester of γ-furfurylidene-α-carboxyethyl-crotonic acid — — — >400 — >400

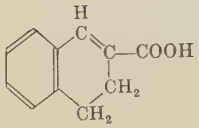
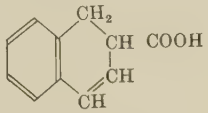
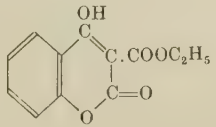
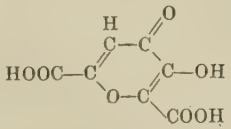
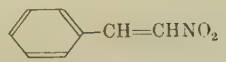
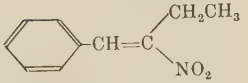
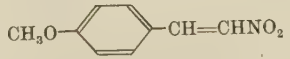
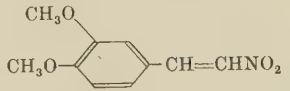
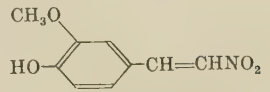
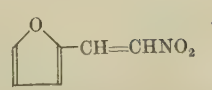
56. 

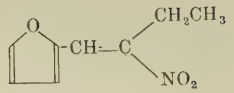
dl-1:4-Dihydronaphthoic acid-(1) — 62·5 — 400 — 400

57. 

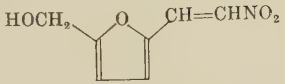
3:4-Dihydronaphthoic acid-(1) — 62·5 — >400 — >400

TABLE 1 (*continued*)

Code no.	Compound	*B. allii* spore germination test (Least conc. (μg./ml.) inhibiting germination)	Agar plate test (Least conc. (μg./ml.) inhibiting growth)	
			F. graminearum	*P. digitatum*
58.	 3:4-Dihydronaphthoic acid-(2)	31·25	400	>400
59.	 *dl*-1:2-Dihydronaphthoic acid-(2)	31·25	80	400
60.	 Methyl ester of 4-hydroxycoumarin-3-carboxylic acid	12·5	400	400
61.	 Meconic acid	>1000	2000	400
	NITROCOMPOUNDS			
62.	 ω-Nitrostyrene	6·25	<3·2	16
63.	 1-Phenyl-2-nitro-Δ¹-butene	250	16	3·2
64.	 4-Chloro-ω-nitrostyrene	7·8	<3·2	16
65.	 4-Methoxy-ω-nitrostyrene	7·8	16	16
66.	 3:4-Dimethoxy-ω-nitrostyrene	1·9	16	16
67.	 4-Hydroxy-3-methoxy-ω-nitrostyrene	0·8	16	16
68.	 1-(Furyl)-2-nitroethylene	62·5	<3·2	<3·2

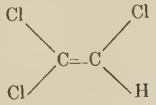
69. 

1-(Furyl)-2-nitrobutene-1 — 500 — 80 — 16

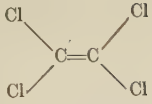
70. 

α-(Hydroxymethyl)-δ-(ω-nitrovinyl)-furan — 62·5 — 80 — 400

MISCELLANEOUS ETHYLENIC COMPOUNDS

71. 

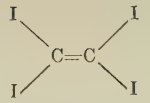
Trichloroethylene — > 500 — > 400 — > 400

72. 

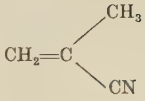
Tetrachloroethylene — > 500 — > 400 — > 400

73. $CH_2{=}CH\ CH_2Br$

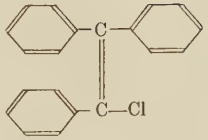
Allyl bromide — 250 — > 400 — > 400

74. 

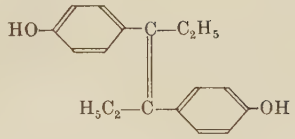
Tetraiodoethylene — 15·6 — 3·2 — 3·2

75. 

Methacrylonitrile — > 1000 — > 400 — > 400

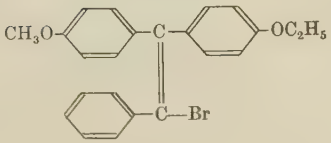
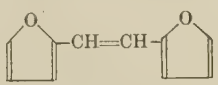
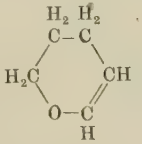
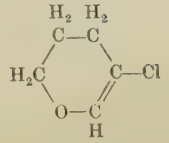
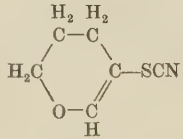
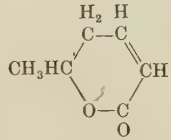
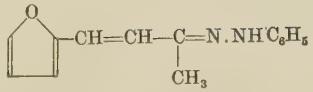
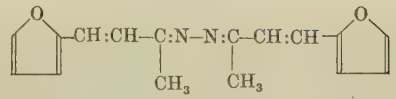
76. 

Triphenylchloro-ethylene — > 500 — > 400 — > 400

77. 

44′-Dihydroxy-αβ-diethylstilbene (Stilboestrol) — > 500 — > 400 — > 400

TABLE 1 (*continued*)

Code no.	Compound	*B. allii* spore germination test (Least conc. (μg./ml.) inhibiting germination)	Agar plate test (Least conc. (μg./ml.) inhibiting growth)	
			F. graminearum	*P. digitatum*
78.	 α-*p*-Anisyl-α-phenetyl-β-phenyl-β-bromo-ethylene	> 500	> 400	> 400
79.	 αβ-Di-(furyl)-ethylene	—	> 400	> 400
80.	 2:3-Dihydropyran	> 1000	> 400	> 400
81.	 5-Chloro-2:3-dihydropyran	> 1000	> 400	> 400
82.	 5-Thiocyano-2:3-dihydropyran	> 1000	400	> 400
83.	 *dl*-$\Delta^{\alpha}\beta$-Hexenolactone	62·5	—	—
84.	 The phenylhydrazone of furfurylidene acetone	—	> 400	> 400
85.	 Furfurylidene acetone azine	—	> 400	> 400

room temperature) is smaller than 10^{-7} and will give largely the meta derivative if K is greater than 10^{-5} gram mols per litre.'

The acidity of the compound AOH can be used as a convenient measure of the attraction of a group A for electrons. Thus nitric acid is a very strong acid and all compounds containing the —C=C—NO_2 grouping are powerful fungicides (Code nos. 62–70). Compounds (Code nos. 5–61) having attached to the ethylenic double bond a group —COO*R*, —CO*R*, —COOH or —CHO show less activity and the corresponding hydroxy compounds are rather weak acids. Although some compounds which contain no groups that are meta-directing in the benzene nucleus do show fungistatic activity in the *Botrytis allii* spore germination test, with one exception none of the compounds show any activity in the tests for inhibition of growth of either *Fusarium graminearum* or *Penicillium digitatum*. This exception is tetraiodoethylene (Code no. 74) and it does not seem possible to attribute the high toxicity of this compound to the withdrawal of electrons from the ethylenic double bond by the iodine atoms. The results with tetraiodoethylene are all the more surprising since the tetraethyl ester of ethylene tetracarboxylic acid (Code no. 40) and tetrachloroethylene (Code no. 71) do not show any similar high activity.

Some of the compounds listed have already been shown to be fungicidal. A large number of nitro-olefines have been shown to be fungicides (Bosquet, Kirby & Searle, 1943). Geiger & Conn (1945) studied a number of compounds containing the —C=C—CO— grouping. Amongst these were benzalacetone (Code no. 8), benzalacetophenone (Code no. 10), furfuralacetone (Code no. 9), and furfuralacetophenone (Code no. 11). Geiger & Conn suggested that the fungistatic action was connected with the ability of these compounds to combine with —SH groups. A similar explanation has been put forward to account for the lachrymatory properties of some ethylenic compounds. According to Dixon & Needham (1946), the lachrymators contain substituent groups (ketone, aldehyde, ester, nitro, etc.) which polarize the adjacent olefinic linkages and render them reactive towards nucleophilic (anionoid) reagents such as compounds containing —SH groups. There is, however, a lack of parallelism between the fungistatic activity and the physiological effects of ethylenic compounds on man (Brian, Grove & McGowan, 1946) and it may be that whilst substituent groups which tend to attract electrons confer general toxicity towards living cells the specific type of toxicity is conditioned by other factors.

REFERENCES

Bosquet, E. W., Kirby, J. E. & Searle, N. E. (1943). Combating pests such as insects and fungi. *U.S. patent* (to E. I. du Pont de Nemours and Co.). No. 2,335,384.

Brian, P. W., Curtis, P. J., Hemming, H. G. & McGowan, J. C. (1946). The production of viridin by pigment-forming strains of *Trichoderma viride*. *Ann. appl. Biol.* **33**, 190.

Brian, P. W., Grove, J. F. & McGowan, J. C. (1946). The fungistatic activity of ethylenic and acetylenic compounds. *Nature, Lond.*, **158**, 876.

Brian, P. W. & Hemming, H. G. (1945). Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. *Ann. appl. Biol.* **32**, 214.

Brian, P. W. & McGowan, J. C. (1945). Viridin: a highly fungistatic substance produced by *Trichoderma viride*. *Nature, Lond.*, **156**, 144.

Brian, P. W. & McGowan, J. C. (1946). Biologically active metabolic products of the mould *Metarrhizium glutinosum* S. Pope. *Nature, Lond.*, **157**, 334.

Dixon, M. & Needham, D. M. (1946). Biological research on chemical warfare agents. *Nature, Lond.*, **158**, 432.

Geiger, W. B. & Conn, J. E. (1945). The mechanism of the antibiotic action of clavacin and penicillic acid. *J. Amer. Chem. Soc.* **67**, 112.

McGowan, J. C. (1936). A new rule for substitution in the benzene nucleus. *Chem. & Ind.* **55**, 607.

(*Received* 29 *May* 1947)

THE FUNGISTATIC ACTIVITY OF ETHYLENIC AND ACETYLENIC COMPOUNDS

II. ESTERS OF HALOGENOFUMARIC ACIDS AND ACETYLENE DICARBOXYLIC ACID

By J. F. GROVE

Imperial Chemical Industries Limited, Butterwick Research Laboratories, The Frythe, Welwyn, Herts

A series of derivatives of acetylene dicarboxylic, halogenofumaric and related acids have been prepared and their fungistatic activities assessed. The lower halogeno- and thiocyanofumarates and acetylene dicarboxylates are highly fungistatic. The mode of action of these compounds is discussed.

Part I (McGowan, Brian & Hemming, 1948) of this series dealt with the anti-fungal activity of a number of substances containing an ethylenic double bond and related the intensity of biological action to the affinity for electrons of the different groups attached to that bond. The present paper is mainly concerned with two series of compounds. One of these, esters of halogeno- and thiocyanofumaric acids, falls into the above general category. The other, comprising esters of acetylene dicarboxylic acid, in which two carboxylic ester groups are attached directly to a carbon-carbon triple bond, constitutes a newly discovered type of antifungal compound.

In Part I, it was found that esters of the isomeric unsaturated dicarboxylic acids, maleic and fumaric acid were appreciably fungistatic and that the *trans* configuration was the more active in this respect. The introduction of a halogen atom into the molecule of an organic compound is often accompanied by an increase in fungistatic activity. We were led therefore, to examine esters of halogeno- and thiocyanofumaric acids, and also a number of solid derivatives, solid substances being more suitable for incorporation in seed dressings.

In the course of this work, the ethyl ester of acetylene dicarboxylic acid, intermediate in the preparation of one of the halogenofumaric esters, was also examined, and the promise shown by this substance encouraged us to investigate the fungistatic potentialities of a number of related compounds.

Experimental

The chemical compounds tested for antifungal activity were prepared as described below:

I. *Carboxylic acids*

Bromofumaric acid, m.p. 182° C., and *chlorfumaric acid*, m.p. 191° C., were obtained from dibromosuccinic and dichlorsuccinic acids respectively by the method of Michael (1895).

Acetylene dicarboxylic acid, m.p. 175° C., was prepared as described by Abbott, Arnold & Thompson (1943); and *propiolic acid*, CH≡C.COOH, b.p. 83°/50 mm., by refluxing an aqueous solution of acetylene dicarboxylic acid (Bandrowski, 1880, 1882).

Chlormaleic anhydride. Equimolecular quantities of chlorfumaryl chloride and chlorfumaric acid heated together at 125° C. for 2 hr. (Perkin, 1888) gave chlormaleic anhydride, b.p. 192°, in good yield.

Dibromofumaric acid. Acetylene dicarboxylic acid (12 g.) in water (15 g.), and liquid bromine (16 g.) were placed in separate vessels in an empty desiccator and the desiccator evacuated. Absorption was complete in 24 hr. and the aqueous solution was then slowly concentrated by standing for some days over sulphuric acid. The colourless crystals which separated were recrystallized from water and dried *in vacuo* over sulphuric acid (15 g.), m.p. 225° C.

1:2-*Dibromacrylic acid*. Propiolic acid (7 g.) in water (8 g.) was allowed to absorb the calculated amount of bromine under the same conditions as described for the preparation of dibromofumaric acid, above. The oil which separated on concentrating the aqueous solution was distilled under reduced pressure and the fraction b.p. 146–50°/6 mm. solidified on cooling yielding dibromacrylic acid, m.p. 82° C. (4 g.).

II. *Esterification of the acids*

Methyl chlorfumarate and the esters of bromofumaric acid were obtained by refluxing the acid with an excess of the appropriate alcohol in the presence of a stream of hydrochloric acid gas.

Ethyl chlorfumarate was prepared from the corresponding dichlorsuccinate by heating with dimethylaniline at 100° C. for 6 hr. (Darzens, 1912).

Thiocyanofumarates were obtained from the corresponding bromofumarates by refluxing for 16 hr. with a 100% excess of potassium thiocyanate in ethanol.

Esters of propiolic acid were prepared by allowing acid and alcohol to stand together for from 4 to 7 days at room temperature in the presence of concentrated sulphuric acid. The remaining acids were esterified by refluxing 6 hr. with an excess of the alcohol in the presence of concentrated sulphuric acid.

β-Diethylaminoethyl esters were obtained by ester exchange with the corresponding diethyl esters.

The crude esters obtained by the above procedures were purified by distillation with the exception of the thiocyanofumarates which were tested in the crude state. The actual boiling range of the fraction submitted for the biological tests is given in column 2 of Table 1. In those cases where the compound has previously been described in the literature, the relevant physical data are given in column 3. All the esters were either colourless oils or colourless crystalline solids. Propiolic esters and the lower esters of acetylene dicarboxylic acid had an irritant action on the mucous membranes of the eyes and nose; the esters of thiocyanofumaric acid possessed the somewhat nauseating odour characteristic of this class of compound.

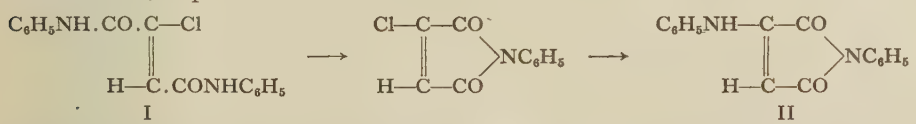
TABLE 1. *Physical characteristics of esters assessed for fungistatic activity*

1 Compound	2 Boiling range of fraction	3 Previous data and reference
Dimethyl acetylene dicarboxylate	83°/5 mm.	102–3°/20 mm. (Moureu & Bongrand, 1920)
Diethyl acetylene dicarboxylate	108–18°/10 mm.	107–8°/13 mm. (Moureu & André, 1914)
Di-*iso*-propyl acetylene dicarboxylate	107°/3 mm.	
Di-*iso*-butyl acetylene dicarboxylate	148°/6 mm.	
Di-β-diethylaminoethyl acetylene dicarboxylate	138°/6 mm.	
Dimethyl bromofumarate	115°/6 mm. (needles m.p. 30°)	m.p. 30° (Anschutz, 1879)
Diethyl bromofumarate	145–55°/11 mm.	135–6°/12 mm. (von Auwers & Harres, 1929)
Di-*n*-butyl bromofumarate	170–2°/8 mm.	
Di-β-diethylaminoethyl bromofumarate	170°/6 mm.	
Dimethyl chlorfumarate	100°/5 mm.	108°/15 mm. (von Auwers & Harres, 1929)
Diethyl chlorfumarate	115–20°/6 mm.	117°/7 mm. (Darzens, 1912)
Diethyl chlormaleate	112°/15 mm.	122°/15 mm. (Thomas-Mammert, 1895)
Ethyl-1:2-dibromacrylate	95°/2 mm.	
Diethyl dibromofumarate	140–50°/2 mm. (prisms m.p. 67° C.)	m.p. 67° (Michael, 1892)
Methyl propiolate	100°/760 mm.	101°/757 mm. (Moureu & Bongrand, 1910)
iso-Butyl propiolate	152°/760 mm.	

III. *Miscellaneous*

Diamidoacetylene, a white microcrystalline powder which decomposed without melting, resulted from the addition of pre-cooled concentrated aqueous ammonia to methyl acetylene dicarboxylate (Moureu & Bongrand, 1920).

Chlorfumardianilide. Chlorfumaryl chloride reacted vigorously with an excess of aniline in ether at −20° C. with the formation of the *dianilide* (I), yellowish needles from ethanol, m.p. 186° C.:



and this compound was converted on heating at 150° C. for a few minutes with an excess of aniline into *phenyliminosuccinanil* (II), a yellow microcrystalline powder, m.p. 232° C. (Michael, 1887).

1-Amino-1:2-diamidoethylene. Methyl bromofumarate was shaken with concentrated aqueous ammonia and the white crystals which separated were well washed with water and dried at 120° C. They decomposed without melting at 185–90° C.

Found N = 30·8 %. $C_4H_7O_2N_3$ requires N = 32·6 %.

Diethylchloroxalacetate. Ethyl chloracetate (22 g.) was added a little at a time to ethyl oxalate (29 g.), metallic sodium (4 g.) and ethanol (8 g.) in dry ether (50 c.c.) and the whole refluxed for 4 hr. The reaction mixture was then made just acid with acetic acid and poured into cold saturated brine. Extraction with ether and its subsequent removal left an oil which was purified by distillation under reduced pressure. Diethylchloroxalacetate, a colourless oil (12 g.), passed over between 140–7°/15 mm.

Ethyl-α-chloroacetoacetate. A technical sample ex B.D.H. was re-distilled and the fraction, b.p. 95°/7 mm., collected. Samples of methyl fumarate and ethyl fumarate were obtained from B.D.H.

Biological evaluation

The methods used for the assessment of fungistatic activity were the same as those described in the first paper of this series (McGowan *et al.*, 1948). The results obtained are shown in Table 2. This table is compiled from the results of a number

Table 2. *Preliminary assessment of fungistatic activity*

Compound	Spore germination test. Least concentration (μg./ml.) inhibiting germination of *Botrytis allii*	Agar plate test. Least concentration (μg./ml.) inhibiting growth of *Fusarium graminearum*	Agar plate test. Least concentration (μg./ml.) inhibiting growth of *Penicillium digitatum*
Dimethyl fumarate	250	400	80
Diethyl fumarate	500	80	400
Bromofumaric acid	>500	>400	>400
Dimethyl bromofumarate	7·8	16	16
Diethyl bromofumarate	31·2	16	16
Di-*n*-butyl bromofumarate	62·5	16	80
Di-β-diethylaminoethyl bromofumarate	>1000	>400	>400
Dimethyl chlorofumarate	31·2	16	16
Diethyl chlorofumarate	500	16	80*
Dimethyl thiocyanofumarate	15·6	80	3·2†
Diethyl thiocyanofumarate	31·2	16*	16
Diethyl chlormaleate	500–1000	400*	80
Diethyl dibromofumarate	500	400	80
Methyl-1-chloracrylate	250	400	80
Ethyl-1:2-dibromacrylate	250	80	16
Diethyl chloroxalacetate	>500	400	400
Ethyl-α-chloracetoacetate	>1000	400	400
1-Amino-1:2-diamidoethylene	250	80	80
Chlorfumardianilide	>1000	>400	>400
Phenyliminosuccinanil	>1000	>400	>400
Acetylene dicarboxylic acid	1000	400	>400
Dimethyl acetylene dicarboxylate	1·9	3·2†	3·2†
Diethyl acetylene dicarboxylate	3·9‡	16*	3·2†
Di-*iso*-propyl acetylene dicarboxylate	62·5	16	16
Di-*iso*-butyl acetylene dicarboxylate	500	80	80
Di-(β-diethylaminoethyl) acetylene dicarboxylate	>550	>400	>400
Methyl propiolate	>1000	>400	>400
iso-Butyl propiolate	>1000	>400	>400
Diamidoacetylene	>1000	>400	>400
Salicylanilide	62·5	80	80

* Only a trace of growth at one-fifth this concentration.
† Lowest concentration tested. ‡ Slight germination at 1·9 μg./ml.

of independent tests but in each case salicylanilide was included as a standard giving identical results on each occasion.

Derivatives of halogenofumaric acids

(III) (IV)

Comparing results for the methyl and ethyl esters of bromofumaric acid (III, $R{=}OR_1$, $R_2{=}H$, $R_3{=}Br$) with the corresponding fumaric esters (III, $R{=}OR_1$, $R_2{=}R_3{=}H$), it can be seen that substitution of the hydrogen atom on one of the α-carbon atoms by a bromine atom is accompanied by a big increase in fungistatic activity. An examination of the results reported in Part I of this series shows that there are few exceptions to the rule that compounds of general type PQC=CRS (PQRS ≠ H) are inactive or of low activity. In accordance with this conclusion substitution of the remaining hydrogen in ethyl bromofumarate by bromine (III, $R{=}OR_1$, $R_2{=}R_3{=}Br$) leads to a decrease in activity. Diethyl dibromofumarate is, in fact, of approximately the same order of activity as diethyl fumarate, and diethyl chloroxalacetate (III, $R{=}OR_1$, $R_2{=}Cl$, $R_3{=}OH$, for the enol form) is less active than diethyl chlormaleate. Ethyl-1:2-dibromacrylate (IV, $R{=}OC_2H_5$, $R_2{=}R_3{=}Br$, $R_4{=}H$) containing only one carboxylic ester group attached to the ethylenic double bond is more active than diethyl dibromofumarate. Methyl-1-chloracrylate (IV, $R{=}OCH_3$, $R_2{=}Cl$, $R_3{=}R_4{=}H$), however, is considerably less fungistatic than dimethyl chlorfumarate (III, $R{=}OCH_3$, $R_2{=}Cl$, $R_3{=}H$) emphasizing the importance of the dicarboxylic ester grouping. Ethyl-α-chloroacetoacetate (IV, $R{=}OC_2H_5$, $R_2{=}Cl$, $R_3{=}OH$, $R_4{=}CH_3$, for the enol form) in which there is no hydrogen atom and only one carboxylic ester group attached to the $\rangle C{=}C\langle$ grouping, is the least active of the ethyl esters evaluated.

As in the case of esters of the unsubstituted unsaturated C_4 dicarboxylic acids, the *trans* configuration is rather more active fungistatically than the isomeric *cis*-form; diethyl chlorfumarate being definitely superior to the isomeric diethyl chlormaleate.

Comparing thiocyanofumarates with the corresponding halogenofumarates (III, $R{=}OR_1$, $R_2{=}H$, $R_3{=}$halogen or —SCN), activity against the particular test organisms used decreases according to the following series, although the actual differences are very small:

$$R_3{:}\text{—SCN} > \text{—Br} > \text{—Cl}.$$

Ascending the homologous series of bromofumaric esters (III, $R{=}OR_1$, $R_2{=}H$, $R_3{=}Br$), activity falls off according to the following scheme:

$$R_1{:}\,CH_3\text{—} > C_2H_5\text{—} > n\text{-}C_4H_9\text{—} > (C_2H_5)_2N.CH_2.CH_2\text{—}.$$

In drawing this conclusion more weight has been given to the results of the *Botrytis allii* spore germination test (dilutions × 2 steps). Bromofumaric acid itself is relatively inactive.

Of the solid derivatives 1-amino-1:2-diamidoethylene (III, $R{=}NH_2$, $R_2{=}H$, $R_3{=}NH_2$) shows moderate activity, whereas chlorfumardianilide (III, $R{=}NHC_6H_5$, $R_2{=}H$, $R_3{=}Cl$) is inactive.

Derivatives of acetylene dicarboxylic acid

$$ROC.C{\equiv}C.COR$$
(V)

The lower esters of acetylene dicarboxylic acid (V, $R{=}OR_1$) are considerably more active than the corresponding halogenofumaric esters. Here again the free acid is inactive and the activity of the esters falls off rapidly on ascending the series:

$$R_1: CH_3{-}> C_2H_5{-}> \textit{iso}\text{-}C_3H_7{-}> \textit{iso}\text{-}C_4H_9{-}> (C_2H_5)_2N.CH_2.CH_2{-}.$$

In contrast to the halogenofumaric series, the diamide (V, $R{=}NH_2$) is devoid of activity. Esters of propiolic acid of general formula $HC{\equiv}C.COOR_1$ are not appreciably fungistatic.

Evaluation in liquid media

Some of the more active halogenofumarates were tested against a wider range of organisms using a closer dilution scale. Dilutions of these substances were made in $\times 2$ steps in 2% malt extract solution. These were dispersed in 25 ml. lots in 100 ml. flasks, inoculated with spores of the test fungi, and incubated at 25° C. The means of five replicate experiments are presented in Table 3.

TABLE 3. *Fungistatic activity of selected thiocyano- and halogenofumarates*

Compound	Least concentration (μg./ml.) preventing growth at 25° C. of				
	Trichoderma viride	*Metarrhizium glutinosum*	*Aspergillus niger*	*Fusarium graminearum*	*Penicillium digitatum*
Dimethyl bromofumarate	>80	20	20	20	5
Diethyl bromofumarate	80	20	20	5	2·5
Dimethyl chlorfumarate	>80	40	40	40	2·5
Diethyl chlorfumarate	80	40	40	10	5
Diethyl thiocyanofumarate	40	40	20	5	2·5
Salicylanilide	80	80	80	40	40

The results of previous tests using 2% malt agar (Table 2) are essentially confirmed, the lower halogeno- and thiocyanofumarates showing activity of a high order against *Fusarium graminearum* and particularly against *Penicillium digitatum*. *Trichoderma viride* is relatively resistant to this class of compound.

DISCUSSION

Anions of organic acids penetrate cell membranes less readily than the unionized molecules (Davson & Danielli, 1943; Hober, 1945). It is well known that the fungistatic activity of an organic acid varies with the *p*H of the test medium (Hoffman, Schweitzer & Dalby, 1939) and that the activity is roughly proportional to the concentration of the unionized molecules present. It is not surprising therefore that acetylene dicarboxylic and bromofumaric acids which are strong organic acids are

inactive. Even at *p*H 3·5 under the conditions of the *Botrytis allii* spore germination test, the acids are present largely in the inactive ionized condition. Esterification of the carboxyl groups is considered to be essential to penetration to the site of action. Increasing the size of the ester grouping and incidentally the lipoid solubility of the whole molecule has a detrimental effect on the fungistatic power of the compound, and, in both the acetylenic and ethylenic series, the methyl and ethyl esters are the more fungistatic. Similar results have been reported in several cases where a homologous series of compounds has been tested for antifungal activity, for example, the xanthates, xanthyl sulphides and thiocarbamates investigated by Davies & Sexton (1946).

Many biologically active compounds act by inhibition of some enzyme reaction in which a metabolite similar in chemical structure to the active compound is involved. Certain C_4 dicarboxylic acids, succinic, fumaric, malic and oxalacetic acids have been shown to be important links in carbohydrate metabolism through the Krebs cycle and the enzyme systems involved, apart from their occurrence in animal tissue, are known to be widely distributed among bacteria, fungi and plants. The suggestion is therefore made that the fungistatic action of the two series of compounds dealt with in this paper may arise from interference with these enzyme systems. Certainly in the acetylenic series, fungistatic activity is only observed when two carboxylic ester groups are attached to the carbon-carbon triple bond, although this is not completely true in the ethylenic series where ethyl-1 : 2-dibromacrylate and methyl-1-chloracrylate are comparable with salicylanilide. Penetration of the ester molecule into the mould cell may be followed by hydrolysis, with liberation of the free acid, which is then adsorbed on the active surface of the enzyme. The enhanced activity of the *trans*- as compared with the *cis*-configuration emphasizes the importance of molecular orientation and supports the view that some reaction at a surface is involved.

The effect of acetylene dicarboxylic, halogenofumaric and related acids on certain enzyme systems involved in the Krebs cycle is being investigated and the results will be presented in a future communication.

Biological activity has been shown (Erlenmeyer, 1938) to be retained by compounds in which sulphur in a ring system has been replaced by the grouping —CH=CH—, and this experimental fact has proved of considerable value in the synthesis of competitive metabolite antagonists such as pyrithiamine (Robbins, 1941). It is of some interest therefore that replacement of —CH=CR_2— in the fumarates and —C≡C— in the acetylene dicarboxylates by sulphur results in dicarbethoxy sulphide EtO.CO.S.CO.OEt, which has been shown (Davies & Sexton, 1946) to have approximately the same order of fungistatic activity.

The author is indebted to Mr J. C. McGowan for much encouragement and advice and to Dr P. W. Brian and Mr H. G. Hemming for the biological assays.

REFERENCES

ABBOTT, T. W., ARNOLD, R. T. & THOMPSON, R. B. (1943). *Organic Syntheses, Collective Volume II*, p. 10. New York: Wiley.

ANSCHUTZ, R. (1879). Über Fumar- und Maleinsäureäther II. *Ber. dtsch. chem. Ges.* **12**, 2284.

VON AUWERS, K. & HARRES, L. (1929). Über die Konstitution der Maleinsauren. *Ber. dtsch. chem. Ges.* **62**, 1685.

BANDROWSKI, E. (1880). Über das Kaliumpropargylat $C_3KHO_2+H_2O$. *Ber. dtsch. chem. Ges.* **13**, 2340.

BANDROWSKI, E. (1882). Über Propargylsaure ($C_3H_2O_2$). *Ber. dtsch. chem. Ges.* **15**, 2699.

DARZENS, G. (1912). Sur les éthers de l'acid dichlorsuccinique et leur isomères stéréochimiques. *C.R. Acad. Sci., Paris*, **154**, 1617.

DAVIES, W. H. & SEXTON, W. A. (1946). Chemical constitution and fungistatic action of organic sulphur compounds. *Biochem. J.* **40**, 331.

DAVSON, H. & DANIELLI, J. F. (1943). *The permeability of natural membranes*. Cambridge.

ERLENMEYER, H. (1938). Zur Kenntnis der Eigenschaften isosterer und strukturähnlicher Verbindungen. VIII. Über die narcotischen Eigenschaften einiger Derivate des 2:4 Dioxothiazolidins und des 2:4 Dioxo-oxazolidins. *Helv. chim. Acta*, **21**, 1013.

HOBER, R. (1945). *Physical chemistry of cells and tissues*. London.

HOFFMAN, C., SCHWEITZER, T. R. & DALBY, G. (1939). Fungistatic properties of fatty acids and possible biochemical significance. *Food Research*, **4**, 539.

MCGOWAN, J. C., BRIAN, P. W. & HEMMING, H. G. (1948). The fungistatic activity of ethylenic and acetylenic compounds. I. The effect of the affinity of the substituents for electrons upon the biological activity of ethylenic compounds. *Ann. appl. Biol.* **35**, 25.

MICHAEL, A. (1887). Researches on Alloisomerism, I. *Amer. Chem. J.* **9**, 180.

MICHAEL, A. (1892). Untersuchungen über Alloisomerie, II. *J. prakt. Chem.* (2), **46**, 229.

MICHAEL, A. (1895). Untersuchungen über Alloisomerie, III. *J. prakt. Chem.* (2), **52**, 301.

MOUREU, C. & ANDRÉ, E. (1914). Thermochimie des composés acétyléniques. *Ann. Chim.* (9), **1**, 126.

MOUREU, C. & BONGRAND, J. C. (1910). Composés propioliques. Cyanacétylène C_3NH. *C.R. Acad. Sci., Paris*, **151**, 946.

MOUREU, C. & BONGRAND, J. C. (1920). Le sous-azoture de carbons C_4N_2. *Ann. Chim.* (9), **14**, 11.

PERKIN, W. H. (1888). Chlorofumaric and chloromaleic acids and the magnetic rotatory powers of some of their derivatives. *J. Chem. Soc.* **53**, 695.

ROBBINS, W. J. (1941). Pyridine analogue of thiamine and the growth of fungi. *Proc. nat. Acad. Sci. Wash.* **27**, 419.

THOMAS-MAMMERT, M. R. (1895). Sur la non-existence de la stéréoisomérie dans les derivés aminobutènedioques. *Bull. Soc. chim. Paris* (3), **13**, 848.

(*Received* 29 *May* 1947)

THE RELATION BETWEEN THE SIZE OF PLANT AND THE SPREAD OF SYSTEMIC DISEASES

II. THE APHIS-BORNE POTATO VIRUS DISEASES

By J. E. van der PLANK

Division of Botany and Plant Pathology, Pretoria

It is an implication of systemic infection by the aphis-borne viruses that potato plants with large haulms should be specially vulnerable to infection, and this implication is supported by evidence in the field.

Haulms are reduced in size by short days, low temperatures, under-nutrition and varietal differences. Certain observations are adduced to show that these factors reduce vulnerability. It is suggested that the transference of the potato from the short days, infertile soils and primitive cultivation on the Andes to the long summer days and productive farming of Europe and North America enhanced its vulnerability to aphis-borne virus diseases: the further effects of changes of temperature and manurial practices in the last century need elucidation.

The general hypothesis, and evidence to support it

Part I of this series (van der Plank, 1947) dealt with the general relation between the size of plant and the rate of spread of infectious systemic disease. Part II deals with the aphis-borne virus diseases of the potato in particular.

In its simplest form our present problem is to determine how large and small plants will react in a field in which aphides—*Myzus persicae* especially—move about. The distribution of aphides, measured in numbers per 100 leaves, or per unit leaf area, can be assumed practically uniform over the field. Large plants with many leaves will harbour more aphides than small plants with few leaves; and one expects they will consequently be more exposed to that one effective transmission of virus which with a systemic disease is all that is needed for complete infection of the plant. That is, one expects that large plants will be more vulnerable to infection than small plants. It is assumed here, of course, that other factors, like variety and age, are constant.

That *Myzus persicae* spreads almost uniformly over the foliage (except for differences in infestation of upper and lower leaves and for differences with the age of the plant, which do not concern us here) and is consequently found in greater numbers on large plants was confirmed experimentally. Plants were divided into three classes: small plants with less than 30 expanded compound leaves, medium-sized plants with from 30 to 50 leaves, and large plants with more than 50 leaves. In one count there was an average of 54 *M. persicae*/100 (expanded) leaves on the small plants, 48/100 leaves on the medium-sized plants, and 46/100 leaves on the large plants; in a second count in another field the respective figures were 150, 169

and 150; in a third 108, 101 and 118; and in a fourth, during a very heavy infestation, 1348, 1312 and 1522. The uniformity of spread seems unaffected by the size of the plant.

The second essential point in the argument, that there should be a difference in vulnerability between large and small plants, has been verified experimentally by Whitehead (1927). He found that with the four varieties, Arran Comrade, Great Scot, Kerr's Pink and Tinwald Perfection, the proportion of *current-season* infections of leafroll was greatest in the largest plants. His conclusion, that 'the larger plants are more likely to act as aphis traps and so become infected whilst less vigorous neighbouring plants remain healthy', is precisely the point we have tried to make, though it is because leafroll is a systemic disease rather than because it is spread by aphides that the connexion between size and infection exists.

The vulnerability of large plants has also been remarked by Kotila (1931), who observed that in Michigan 'it is not an uncommon experience in looking over seed plots planted with hill-selected potatoes to find that a higher percentage of virus diseases is present than in the general field'.

Parallel instances are observed with other crops and diseases. For example, Watson, Watson & Hull (1946) remark that observations on the yellows virus disease of beet suggest that very small plants tend to escape infection 'because they are screened or overshadowed by their larger neighbours'.

It may be interpolated here that there have been many uncritical statements about the value of maintaining productivity of potato stocks by selecting seed from the largest plants. As Whitehead pointed out, the method is likely to be of value only where aphides are scarce; where aphides are abundant and the proportion of diseased plants infected during the current season is high, the selection of the largest plants is positively harmful.

There is another form in which the problem is met. Where aphides occur, there is fairly general evidence of a greater rate of infection in potato crops which grow vigorously. Two examples are worth quoting, though evidence of this sort is not so clear-cut as in Whitehead's experiments. A considerable amount of seed is grown in the western states of the United States, especially Nebraska, Montana, Wyoming, Colorado and Idaho, generally without irrigation. On irrigated fields the growth of haulm is much more vigorous, and experience is that virus diseases spread more quickly. Edmundson (1940) explains the matter thus: 'Disease is spread more rapidly because of rank vine growth and contact of one plant with another.' In ware-growing districts of England, Gregory (1943) noticed that on several fields where the haulm remained small and compact the percentage of plants affected with leafroll did not increase after July, whereas on certain other fields remarkable for the immense growth of haulm (6 to 7 ft.) the largest increases of leafroll were recorded in August and early September. Gregory comments that 'it seems likely that a sprawling and densely interlacing foliage would greatly facilitate the movement of wandering aphides, such as the apterae of *Myzus persicae*, from plant to plant'.

Both Edmundson and Gregory recognize the importance of size, although they see it not peculiarly in relation to systemic disease, but primarily as a matter of contact. Contact is undoubtedly a factor of great importance with wandering aphides on a crop like potatoes with interlacing leaves, but has not the significance of size itself which affects the spread of all systemic diseases,* whether they be spread by crawling insects or not, in all crops, whether they have the sort of leaves which interlace or not.

The effect of length of day

In dealing with size we are concerned with the part which receives infection (see Part I). With aphis-borne virus diseases of potato this is the haulm, which for present purposes we may regard as the gross entity of lamina, petiole, stalk and inflorescence.

The growth of the haulm is sensitive to the length of day. Some figures of Hackbarth (1935) for South American clones illustrate this. With 'long-day varieties' (varieties which produce the maximum yield per stool in long days) 1 g. of haulm (weighed air-dry) produced during the course of the growing-season an average of 2·5 g. of tubers when the days were the normal long days of the German summer, and 17·1 g. when the day was artificially reduced to 12 hr. With short-day varieties the corresponding figures were 0·8 g. and 11·0 g. That is, in short days a given weight of tubers can be produced by one-fourteenth to one-seventh the mass of haulm which is needed in long days; or, to put the matter another way, for any given abundance of aphides, measured in terms of numbers per 100 leaves or some other unit of size of haulm, a crop may be produced in short days with far fewer aphides per plant and, presumably, far less chance of infection of any individual plant. Differences of the same sort have been obtained with commercial varieties. Werner's (1940) Fig. 5 is a good graphical illustration of the great difference which length of day makes on the distribution in Triumph potatoes of dry weight between haulm and tuber.

In South Africa length of day has been a dominant factor in maintaining the health of potatoes, though there was no conscious realization of the fact. The great bulk of South African potatoes are produced during the fairly long days of summer, one crop being grown a year. Only in the most favourable circumstances has it been possible to keep stocks healthy for many generations on this system of cropping. Less commonly, two crops are grown a year, one in spring and one in autumn, both in roughly equinoctial conditions; and to this system of cropping almost all the instances of the successful cultivation of seed over long periods have been confined.

To give details, there are several records of stocks being maintained for long periods in the Knysna-George-Great Brak River area of the southern Cape coast.

* It is appropriate to quote the relevant theorem: The spread of an infectious systemic disease increases with the size of plants. . . other factors being constant. Here we have some other factor, the intimacy of contact between plants, which is not constant but varies in a way so as to accentuate the effect of size itself. For many practical purposes it is unnecessary to distinguish the effects of the two factors, but simply to treat large plants as specially vulnerable to infection.

The varieties involved are mainly Arran Chief and Evergood, vigorous stocks of the latter variety being not infrequent although (according to local evidence) they have been grown there for 40 years, which is 80 generations. The climate is fairly cool and moist by South African standards, and two crops are grown a year. In one instance, Arran Chief has been grown for many years in the eastern Cape, near Grahamstown, under irrigation and with two crops a year. This is well out of the coastal area. A stock of Flourball has been grown in perfect health under irrigation in the Byrne valley, near Richmond, Natal, for about 20 years. The valley is fairly hot and dry, but nothing exceptional by South African standards. Two crops a year are planted. Some Up-to-Date in good health were found in Woodbush Mountain. They could be traced beyond reasonable doubt to an importation in the 1920's. The climate is cool and misty, and the grower stated that he generally avoided planting in summer to escape blight. (Blight is prevalent on the mountain in crops planted about November, but much less so in crops planted early, from July to September, or late, from the end of January onwards.) There was a flourishing little potato industry (now dying out because of eelworm) at Petrusburg, in the Free State, old stocks of Early Rose being favoured. The climate is hot and dry, even by South African standards, and two crops a year are grown. Finally, to conclude the evidence about the system of two crops a year, Mundy (1923) records that in Rhodesia one grower raised over twenty successive crops from the original seed by this method. All these records are for the keeping of seed by farmers, without special guidance; and are almost all for areas where there is no special isolation and protection from diseased fields in the neighbourhood. Against these records, we can set only two for summer-grown potatoes with one generation a year. An instance of Up-to-Date being kept healthy for years was traced to the Underberg district at the foothills of the Drakensberg. Potatoes of various varieties, many of them very old, are grown at Dullstroom at an altitude of nearly 7000 ft. on the Steenkampsberge. The climate here is bleak and cold, cool even by N.W. European standards, and quite unusual for South Africa.

The writer is satisfied that these records are virtually complete;* he took particular trouble to track down stocks of potatoes which had been grown for many years. Considering that the vast bulk of potatoes are grown in South Africa in summer, with one generation a year, the instances of the survival of stocks on the system of two crops a year are quite disproportionately frequent. At the start, the writer could find no explanation for this. A climatic explanation seems ruled out; with two crops a year potatoes have survived in cool, moist conditions at the coast, in hot, dry plains in the interior, in dry river valleys and on misty mountains. Scarcity of aphides was also found to be an unpromising explanation. The Knysna-George-

* The old potato varieties of Basutoland, which grow in summer, are not included, because there is no evidence that they stay healthy for long when planted in rows. The Basuto potatoes are characteristically grown in a state of great dispersion, mixed with maize or other plants; and when planted in patches in rows often become severely infected with leafroll and mosaic. The health of the old stocks has therefore probably nothing to do with our present discussion.

Great Brak River area has been most fully studied, because of its importance as a seed area, and the average of a number of counts made in successive years on plants which had come into flower was 53 *Myzus persicae*/100 mature compound leaves. This is not the maximum reached at the peak of infestation; because the planting season extends over many weeks, counts have been made on plants on which the flowers have opened, irrespective of whether infestation was at its peak or not. This average for the Knysna-George-Great Brak River area is appreciably higher than the figures usual for Pretoria in summer, but, by contrast with the coastal area, the spread of virus disease in the lush growth of fields near Pretoria in summer is very rapid. Finally, the infestation of sprouts was checked. When crops are grown in summer with one generation a year the seed often develops long sprouts which are not so common when two crops have to be fitted into a year; and the possibility suggested itself that aphis infestation of the sprouts might account for the rapid degeneration of stocks grown once a year. But a careful check showed that aphides were not common, and that stocks went down rapidly to leafroll in summer areas even when not a single aphis could be found on the sprouts.

At this stage the writer became interested in photoperiodism. All the available evidence of the literature indicates that short days reduce the growth of haulm and that small haulm growth reduces current-season infection by aphis-borne virus diseases. It follows that, other things being equal, short days should reduce current-season infections. This explains the findings about two crops a year in South Africa. But this tallying of expectation with observation does not imply that the assertion of the benefits of short days in checking the spread of aphis-borne virus diseases is based only on the observations of the survival of potato stocks in the Union and Rhodesia.

With 'long-day varieties' there is some risk of a reduced yield if crops are taken during short days, but, with most varieties, shortening the day-length has no deleterious effect on yield (Driver & Hawkes, 1943). Hence shortening day-length is likely to be a more practical disease-control measure than other methods of reducing haulm, such as restricting manures and fertilizers.

The possibilities of taking short-day crops are not restricted to the tropics and sub-tropics. In Mediterranean climates, at least one short-day crop a year is possible. In continental climates at high latitudes, it is difficult to take a short-day crop because of the steep change of temperature in spring and autumn. Spring and autumn crops must either end or begin in high temperatures, which are likely to destroy any advantage to be gained from short days.

The effects of altering day-length and other practices that affect size of haulm may help to explain the history of potato virus diseases. On the equatorial Andes the days are short and, in primitive peasant cultivation at any rate, manures scanty and fertilizers absent. The growth of haulm has therefore the minimum of stimulation, especially at high altitudes where temperatures are low. Bringing the potato to the long summer days of Europe, and liberal application of manures and fertilizers have,

by producing luxuriant foliage, created a vulnerability to systemic disease quite unknown on the high Andes. The European and North American potato virus problem is thus to a great extent the result of location at high latitudes and of productive agricultural practice.

The effect of temperature

Low temperatures similarly reduce the size of the haulm (Driver & Hawkes, 1943) without necessarily reducing the yield. The effect on yield of temperature reduction depends on whether the temperature is above or below the optimum for tuber formation (about 60–64° F. with long days).

The reduction of haulm growth by low temperatures, quite apart from any effect which low temperatures have on the number and movement of the aphides themselves, is a contributory reason for growing seed in Scotland rather than England, or in Maine rather than in states to the south. Quanjer (1925) attributed the prevalence of virus diseases in Holland, as compared with Scotland, to the more luxuriant growth of the plants.

To some extent the temperature at which crops are grown may be changed by changing the date of planting. In England, for example, an advance of the date of emergence by 1 week will lower the average temperature of the first 2 months of growth—the critical period in the shaping of haulm growth—by about 1° F., quite an appreciable change in the scale of temperatures and one which should be of some value in reducing current-season infection. That early planting does in fact reduce infection of leafroll and mosaic has been shown by Folsom (1940) and Simpson (1940) in Maine. It is likely that the reduction must be ascribed to several factors in combination, rather than to a single factor. Simpson ascribes it to the possibility of earlier roguing of diseased plants and of earlier harvesting of crops.

A change of temperature has probably been of some significance to the spread of virus disease in the past. A hundred years ago temperatures were lower. Since 1850 there has been a world-wide recession of glaciers (Matthes, 1939). During the 30-year period ending 1870, the mean temperature in N.W. Europe and at Cape Town was about 1° F. lower than in the 30-year period ending 1938, and about 2° F. lower in the United States (Kincer, 1940).

The effect of soil fertility

Small plants grown on poor soil are likely to have some resistance to current-season infection. As fertility increases, this resistance will dwindle. Reference has already been made to the observations of Gregory (1943) on this point. In Holland, Janssen (1929) found that the current-season spread of mosaic in the varieties Fontein, Eigenheimer and Zeeuwsche Blauwe, of leafroll in Thorbecke and Deodara, and of both diseases in Schoolmeester was less in sandy soil without nitrogenous fertilizer than when nitrogen was applied. It is reasonable to suppose that at least one effect of the nitrogen was greatly to stimulate the growth of the plants. In Maine, Ross

(1946) has shown that the spread of leafroll increases with increasing rate of fertilizer application. Better nutrition, in so far as it increases haulm growth, carries with it the danger of greater infection.

Secondly, fertilizer mixtures can be altered, especially in nitrogen content, so as to alter the ratio of tubers to haulm. Mixtures which give adequate yields with the minimum of haulm need investigation in seed production.

Thirdly, there may be specific effects of fertilizers on the resistance of the potato or the multiplication of the aphides which have nothing to do with the size of haulm. The results of Janssen (1929) with potatoes in the glasshouse indicate a specific effect of nitrogen, and those of Ross (1946) a specific effect of phosphorus, on susceptibility to leafroll.

Such support as is available from the literature of fertilizers indicates a relation between size and current-season infection. Moreover, soil fertility may have had considerable historical influence. There is fair, but necessarily incomplete, evidence that in the first half of last century about twice as many potato sets were planted per acre in England as now. This indicates that plants were smaller. This might perhaps be ascribed to slightly lower temperatures and the absence of a great supply of artificial fertilizers. The question is, did the small size and close spacing of the plants contribute towards keeping stocks healthy at a time when there was no great organized seed industry and virus infection was controlled largely by raising seedlings, a tedious procedure not adapted to renewing stocks every year or every other year as is now necessary, or was the small size an indication of prevalent virus disease? There is some evidence, also necessarily incomplete, that yields have been high in the past. Wallace (1941) quotes Arthur Young to show that yields in Lincolnshire were higher at the end of the eighteenth century than they are now, and there is reason to believe that yields are now no greater than they were in 1845. (See editorial comment in the *Gardeners' Chronicle*, 12 January 1946.)

The effect of varietal size

The chief difference in size is between early and late varieties. Early varieties are smaller, and for that reason among others should be less vulnerable to systemic infection. That is the general experience in England, although, the inherent susceptibility of varieties varying so greatly, one may expect, and does find, occasional exceptions, like Arran Pilot.

Factors of number of plants per acre, of early planting, and of early harvesting are also involved.

The nature of the evidence

It is deduced that the rate of current-season infection by a systemic disease should vary with the size of the part of the plant which receives it: in our present discussion, the haulm. This deduction is supported by the experiments of Whitehead and others.

Secondly, the deduction is applied to problems of length of day, temperature,

fertilizers, and varietal differences in size. The evidence of each factor, considered singly, may seem flimsy. That the successful maintenance of stocks in South Africa has largely depended on planting two crops a year cannot be indisputably ascribed to the reduction of haulms by short days; that the aphis-borne virus diseases spread less rapidly in colder climates or with earlier planting of crops must be attributed to several factors, of which the reduction of size of haulm by lower temperatures is only one; that increased applications of fertilizer increase the spread of infection is not altogether a question of size; and the lesser vulnerability of early varieties is probably not simply a result of the smaller haulms these varieties usually have. But the evidence all fits, and supports the deduction that potato plants with large haulms are specially vulnerable to aphis-borne infections.

REFERENCES

DRIVER, C. M. & HAWKES, J. G. (1943). Photoperiodism in the potato. *Imp. Bur. Plant Breed. & Genetics Publ.* 36 pp.

EDMUNDSON, W. C. (1940). Potato production in the Western States. *U.S. Dept. Agric. Fmrs' Bull.* 1843, 27 pp.

FOLSOM, D. (1940). Potato virus disease studies with tuber-line seed plots and insects in Maine 1927–1938. *Maine agric. Exp. Sta. Bull.* **410**, 215–50.

GREGORY, P. H. (1943). The spread of potato virus disease in the field. *Ann. appl. Biol.* **30**, 104.

HACKBARTH, J. (1935). Versuche über Photoperiodismus bei südamerikanischen Kartoffelklonen. *Züchter*, **7**, 95.

JANSSEN, J. J. (1929). Invloed der bemesting op de gezondheid van de Aardappel. *Tijdschr. PlZiekt.* **35**, 119.

KINCER, J. B. (1940). Relation of recent glacier recessions to prevailing temperatures. *Monthly Weather Rev.* for June, pp. 158–60.

KOTILA, J. E. (1931). Experiments with the tuber index method of controlling virus diseases of potatoes. *Michigan agric. Exp. Sta. Tech. Bull.* **117**, 26 pp.

MATTHES, F. E. (1939). Report of committee on glaciers. *Amer. geophys. Union Trans.* **20**, 518.

MUNDY, H. G. (1923). *Sub-tropical agriculture in South Africa with special reference to Rhodesia.* Salisbury (Rhodesia), p. 197.

QUANJER, H. M. (1925). Vergelijking tusschen den Gezondheitstoestand van scotsche en noord-hollandsche Pootaardappeln. *Tijdschr. PlZiekt.* **31**, 7.

ROSS, A. F. (1946). Effect of fertiliser practice on spread of leafroll. *Maine agric. Exp. Sta. Bull.* **442**, 128.

SIMPSON, G. W. (1940). Aphides and their relation to the field transmission of potato virus diseases. *Maine agric. Exp. Sta. Bull.* **413**, 189.

VAN DER PLANK, J. E. (1947). The relation between the size of the plant and the spread of systemic disease. I. *Ann. appl. Biol.* **34**, 376.

WALLACE, J. C. (1941). Potatoes. *Min. Agric. Bull.* 94.

WATSON, M. A., WATSON, D. J. & HULL, R. (1946). Factors affecting the loss of yield of sugar-beet caused by beet yellows virus. I. Rate and date of infection; date of sowing and harvesting. *J. agric. Sci.* **36**, 151.

WERNER, H. O. (1940). Response of two clonal strains of Triumph potatoes to various controlled environments. *J. agric. Res.* **61**, 761.

WHITEHEAD, T. (1927). Experiments on the control of potato leafroll. *Welsh J. Agric.* **3**, 169.

(*Received* 13 *May* 1947)

VIRUS DISEASES OF CACAO IN WEST AFRICA

III. TECHNIQUE OF INSECT TRANSMISSION

By A. F. POSNETTE and A. H. STRICKLAND
West African Cacao Research Institute, Tafo, Gold Coast

Experiments on the technique of insect transmission of the cacao virus 1 A (swollen-shoot) are described. This virus is unique in being transmitted by mealybugs (Coccoidea) and the experiments show that all stages of *Pseudococcus njalensis* Laing and of *Ferrisia virgata* Ckll. are vectors. These insects become infective after feeding for less than 4 hr. on the infected plant and transmit after less than 3 hr. on the test plant. The virus is non-persistent in the vector after 3 hr. test-feeding. The vectors can collect virus from either leaf, green shoot, bark or pod; the young symptom-bearing leaf is the best site for infection-feeding and the cotyledon of the bean for test-feeding.

1. Introduction

The viruses infecting cacao in West Africa and causing the various forms of swollen-shoot disease (Posnette, 1947*a*) are, as far as the authors are aware, the only viruses proved to be transmitted by mealybugs. This paper describes experiments which led to the development of a satisfactory technique for virus transmission with these unusual vectors.

The search for the vectors was commenced by the senior author in 1940 and continued by G. S. Cotterell in 1941–2. In the course of this work, two species of Heteropterous insects, fifteen species in eight families of Homoptera, and the Thysanopteran, *Selenothrips rubrocinctus* Giard, were tested as vectors. The results obtained have already been published (Posnette, 1941; Cotterell, 1943); transmissions of the cacao virus 1 A were obtained with the mealybugs (Coccoidea) *Pseudococcus njalensis* Laing, and *Ferrisia virgata* Ckll., and apparently also with the psyllid, *Mesohomotoma tessmanii* Aulm and the aphid, *Toxoptera coffeae* Nietner (= *T. aurantii* Boy.).* These results were treated with due reserve because no other mealybug-transmitted virus disease was known, apart from the unconfirmed reports by Olitsky (1925) and Elmer (1925), and it seemed strange that one virus should be transmitted by members of three super-families of insects.

In 1943, H. E. Box (1945) carried out a series of transmission tests with aphids, psyllids and mealybugs. He included in his experiments a third species of mealybug, *Pseudococcus citri* Risso, a Ricaniid bug, and the cacao thrips (*Selenothrips rubrocinctus*). Box obtained transmissions with all three species of mealybugs, but with none of the other insects, so that when the writers commenced vector investigations in 1945, the evidence on the virus transmitting ability of the aphid (*Toxoptera coffeae*) and the psyllid (*Mesohomotoma tessmanii*) was conflicting.

* We are indebted to Dr W. J. Hall for confirming the identity of these Homoptera.

2. Confirmation of vectors

Using the virulent cacao virus 1A, an experiment was set up to compare *Pseudococcus njalensis* and *Ferrisia virgata* as vectors with *Toxoptera coffeae* and *Mesohomotoma tessmanii*.

The insects were collected from healthy cacao in the field, or, in the case of some of the *Ferrisia virgata* material, from natural infestations on *Leucaena glauca* L.

In the laboratory, the insects were transferred with a fine brush from the field hosts to a Petri dish containing a moist filter paper. When fifty insects had been placed in a dish, it was examined under a binocular microscope and any contamination, such as mealybug 'crawlers' in a psyllid dish, was removed. The dish was then taken to an insect-free house and the insects were transferred to a graft-infected cacao seedling showing virus symptoms, the 'source plant'.

After the appropriate period on the source plant, the insects were removed to a Petri dish, taken to a neighbouring insectary containing only healthy cacao seedlings, and transferred to the appropriate test plants. A separate insectary was used for each species of insect.

Pseudococcus njalensis and *Ferrisia virgata* were fed for 2, 4, 6, 8, 10, 12 and 14 day periods on the source plants. *Toxoptera coffeae* was fed for 2, 4 and 6 day periods, and *Mesohomotoma tessmanii* for 2, 4, 6 and 8 day periods. The mealybugs fed for a more extended period than the aphids and psyllids as their nymphal life was longer. All insects were allowed to remain on the test plants as long as they survived up to a maximum of 60 days after the last infestation, when the plants were sprayed with a 1% nicotine and soft soap emulsion.

A 'bombardment' technique was employed whereby each test plant was infested several times with insects that had fed for varying periods on the source plants. Owing to the depression of growth by the virus in the source plants and consequent lack of young leaves on which psyllids could feed, fewer psyllids per plant were transferred than mealybugs.

As the results (Table 1) agreed with Box's conclusion that the cacao aphid and the cacao psyllid do not transmit virus 1A under these conditions, it was decided to concentrate on the two species of mealybug known to be vectors. The remainder of this paper deals with the experimental results obtained while working out a satisfactory technique for using mealybugs as vectors.

Table 1. *Vector test*

Vector	Total virus-fed insects used	Number of test plants	Transmissions
Pseudococcus njalensis	1018	20	4
Ferrisia virgata	2270	20	9
Toxoptera coffeae	584	10	—
Mesohomotoma tessmanii	167	10	—

3. Manipulation of mealybugs

Certain manipulative difficulties, described below, resulted from the predominantly sessile habits of the adult female mealybugs, *Pseudococcus njalensis* in particular:

(*a*) *Feeding.* The tubular mouth-parts of a mealybug may be inserted to a depth of 1·5 mm. into the plant tissues, and Carter (1945) has shown that the insertion may not be direct, but that the stylets may take an irregular course in the host tissues, presumably until the tip comes in contact with an attractive feeding site. This may lead to the insects being damaged during their removal from field host and source plants, and was presumably one cause of the low (20%) establishment of *Ps. njalensis* in the vector-test experiments.

(*b*) *Locomotion.* When mealybugs had been induced to withdraw their mouth-parts, they were difficult to re-establish, as their tendency was to walk or fall off the new food plant.

(*c*) *The size of the young stages.* First instar 'crawlers' are less than 0·4 mm. long at birth and therefore hard to see with the naked eye and difficult to handle as vectors, especially when resting underneath a mature female. Difficulty was encountered in removing all young nymphs from the mature females before establishing the latter on experimental plants.

Methods have been found to overcome or avoid these difficulties, with the result that the original 20% rate of establishment on receptor plants has been raised to 100% and the percentage of positive transmissions from 15 to 70% or more.

A satisfactory technique developed in the course of the experiments detailed in the succeeding pages, is as follows:

Collection

Insects are collected as colonies on healthy cacao pods which are brought to the laboratory, tapped several times on the bench, and put aside for a few minutes. This treatment disturbs a large proportion of the young adults and nymphs, causing them voluntarily to withdraw their mouth-parts and to search actively for a more peaceful resting place. When walking, the insects are easily transferred on a dry brush to a Petri dish or watch-glass.

All stages of *Ps. njalensis* are negatively phototrophic, and this response can be utilized for the bulk removal of these insects from cacao pods. The pods are placed in a Berlese-Tullgren funnel fitted with a 40 W. electric bulb, and a collecting tube covered with black paper is sealed to the base of the funnel with Plasticine. A large proportion of the nymphs and young adults migrate into the tube while the majority of the mature insects, which are less suitable for transmission experiments, remain on the pod after 18 hr.

Infection-feed

The insects are transferred with a brush to the lower surface of the young leaves ('flush') of the source plant. To prevent the insects from falling or walking off the

plant and to hold the leaves in position, a paper cone is fixed with Plasticine around the stem about 2 in. below the terminal bud.

When large numbers of infective insects are required and the feeding time on the source plant is immaterial, mealybugs can be transferred direct to the receptor plants from pods off infected trees. Whether they are collected from healthy or infected trees, it is part of the experimental technique to transfer a proportion of the insects direct from the field host to healthy cacao plants as a check on their infectivity.

Test-feed

After the appropriate infection-feeding time, the vectors are removed from the source plant with a brush and transferred to test plants previously fitted with paper cones and kept in an insect-proof house (40 mesh 35 s.w.g. gauze). Gently brushing and lifting the insects accelerates the retraction of their mouth-parts, which are more readily withdrawn from the flush leaves than from pods or stems. After the appropriate feeding time, the test plants are disinfested by hand and then sprayed to run-off with a 1% nicotine and soft soap solution.

This method of test-feeding on cacao plants was superseded first by the use of germinating seedlings on which the expanded cotyledons form a convenient feeding-site, and afterwards by the bean-feeding technique which is described later in this paper.

4. Infection-feeding experiments

The shortest feeding-periods on the source and test plants necessary for infection to occur, the insect stages capable of transmitting the virus, and the feeding positions on the source plants from which the vectors can obtain the virus were investigated. In all these experiments, the plants used for infection-feeding were either those infected with virus 1 A by *Pseudococcus njalensis* in the vector test, or cacao seedlings infected by grafting from them.

Infection-feeding time

All stages of *Ps. njalensis* were used with four feeding periods on the source plants, and with an indefinite period on the test plants. Each receptor plant was infested once only, with from four to fourteen insects, and if establishment was unsatisfactory, the plant was discarded. The results, given in Table 2, show that vectors feeding on an infected plant for less than 4 hr. can pick up sufficient virus to infect a healthy plant.

Position of infection-feeding

Three feeding positions on the source plants were used, namely 'flush' leaves showing virus symptoms, the green shoot including the growing point, and the hardened bark on the stem. Each receptor plant was infested once with four to fourteen insects including all stages of *Ps. njalensis*. The results, given in Table 3, show that vectors feeding on any part of an infected plant can obtain the virus and

transmit it to a healthy receptor plant, but that leaves can provide a higher rate of transmission.

TABLE 2. *Duration of infection-feed*

Infection feed (hr.)	Number of test plants	Transmissions
4	24	2
8	18	1
24	30	5
48	26	7

TABLE 3. *Position of infection-feed*

Position on source plant	Number of test plants	Transmissions
Leaves	39	11
Shoot	28	3
Bark	26	1

It has been found that mealybugs taken from pods on naturally infected trees, especially those pods showing virus symptoms, are infective. Mealybugs transferred from fourteen pods to ninety-six seedlings, five insects per plant, resulted in an average of 15% transmission; from fifty-six pods, ten mealybugs per plant infected 23% of 130 seedlings.

Infectivity of insect instars

Pseudococcus njalensis normally undergoes three moults before becoming adult. As the first- and second-instar nymphs cannot be accurately distinguished unless mounted for microscopical examination, these two stages were grouped as 'crawlers', and were compared with third-instar nymphs, and young adults. Each test plant was infested once, the 'adult' plants with four to five insects, the 'nymph' plants with five to eight insects, and the 'crawler' plants with ten to fourteen insects. The results, given in Table 4, show that all stages of the insect can transmit the virus; from this preliminary test it would seem that the crawlers are the most efficient vectors.

Proportion of infective insects

At the conclusion of these and of other experiments to be described in the next section of this paper, only fifty-four out of a total of 228 test plants had become infected. The reason for this low (24%) rate of transmission was investigated in an experiment designed to show what percentage of insects became infective after 24 hr. infection-feeds on flush leaves showing virus symptoms.

Adults and third-instar nymphs of *Ps. njalensis*, which appeared to be feeding, were removed from the source plant with the greatest care, and one insect was transferred to each of 100 cacao seedlings and allowed to feed for 7 days. Their movements on these test plants were watched and their feeding-sites recorded. Insects were alive after 3 days on sixty-eight plants, of which seven became infected. If these sixty-eight plants only are considered, on the assumption that the

insects on the remaining thirty-two did not feed, the experiment indicates that only 10% of the vectors were infective.

5. TEST-FEEDING

Feeding time on test plant

After a 48 hr. infection-feed, five to fourteen mealybugs were transferred to each of thirty test plants (five per treatment) and allowed to feed for periods of 3, 6, 18, 48, 96 and 192 hr., after which the insects were transferred to a second series of test plants for a 7-day feeding period. None of the plants in this second series became infected, indicating non-persistence of the virus in the vector. The results, given in Table 5, show that mealybugs can infect a plant with the virus while feeding for less than 3 hr.

TABLE 4. *Instar test*

Insect instars	Number of test plants	Transmissions
Crawlers	13	4
Nymphs	33	4
Adults	52	7

TABLE 5. *Duration of test-feed*

Feeding time on test plants (hr.)	Number of test plants	Transmissions
3	5	3
6	5	1
18	5	—
48	5	2
96	5	2
192	5	5

This experiment was repeated, using five insects per plant and shorter feeding periods. After 18 hr. starvation to encourage immediate feeding, the insects were allowed either 1 hr. or 4 hr. on the source plants and then ½ hr., 1 hr. or 3 hr. periods on the first series of test plants before being transferred to the second series. Only two transmissions were obtained from sixty test plants, both after the 4 hr. infection-feed. One in the first series of test plants showed that transmission can be completed in a 7 hr. period composed of 4 hr. infection feeding and 3 hr. test-feeding. The second transmission occurred in the second series of test plants after a ½ hr. period in the first, and in the light of subsequent experiments it is probable that no feeding occurred on the first test plant.

Latent period in large seedlings

In all the experiments described so far, relatively large cacao seedlings (10–12 months old) growing in pots were used as test plants. The appearance of symptoms depends on the production of a flush of new leaves, and with such plants the latent

period between the time of infestation and of the appearance of new leaves with symptoms may extend over several months, thus delaying further experiments.

An experiment was therefore designed to test the effect of the stage of growth at the time of infestation on the latent period in year-old seedlings.

After 48 hr. on the leaves of the source plant, vectors were transferred to ten plants in each of the following stages of growth:

Incipient flush (IF). New leaves not more than $\frac{1}{2}$ in. long.

Young flush (YF). New leaves up to 4 in. long, still red and flaccid.

Hardening flush (HF). New leaves of full length, light green.

Out of flush (OF). Leaves dark green and hard.

All the vectors were fed on the growing points of the test plants. In addition to this experiment, the degree of flush was noted for the thirty plants infested in the 'Feeding time on test plant' experiment detailed above; the combined results are given in Table 6.

Table 6

Degree of flush	Plants infested	Number of transmissions	Range of latent period (days)	Mean latent period (days)
IF	14	5	26–46	36
OF	26	11	17–44	32
HF	16	6	21–69	31
YF	14	4	24–33	28

In the last column of Table 6, the only significant difference at the 5% point is between IF and YF. This is to be expected, since a YF plant would harden off and flush again sooner than an IF plant infested at the same time. It will be noted that one OF plant showed symptoms in 17 days; another in the same series produced symptoms in 18 days.

Cotyledon seedlings

The results of the previous experiment were disappointing in that the latent period was still variable even between plants infected in the same stage of flush. At least part of this variation was due to differences in the frequency of flushing of individual plants. In order to minimize this source of variation, and also to economize in greenhouse space, young seedlings between 3 and 5 weeks old were tried. At this stage flushing is more regular, the plants can be grown in small containers such as bamboo pots or cigarette tins, and the cotyledons form a convenient site on which the mealybugs feed readily.

Infection-feeding was carried out as in the previous experiments. The insects were allowed to feed for 48 hr. before being transferred five to each of ninety cotyledon seedlings, of which twenty-eight became infected. The mean latent period was 31 days, and although this is not appreciably shorter than in previous experiments, greater uniformity was noticeable.

Bean-feeding

The experiments described so far had indicated that only about 30% transmission could be expected with five mealybugs per plant. It was important therefore to reduce the space occupied by each test plant to a minimum so that larger numbers of plants could be used in each experiment, and also to develop a technique in which the insects could be kept under close observation during the recording of precise feeding times. Mealybugs had fed satisfactorily on the cotyledons of germinating beans, and it seemed probable that they would feed on the beans themselves.

Cacao beans have proved the most satisfactory test plants if the testa and one cotyledon are removed so that the mealybugs can feed in the inner folds of the remaining cotyledon (Posnette, 1947*b*). (*Ferrisia virgata*, *Pseudococcus njalensis*, *Ps. citri* and *Ps. concavocerarii* James, have been tried and all feed readily.) A bean fits conveniently into a block watch-glass,* which with a glass cover forms an insect-proof cage. Should the insects migrate off the bean, they can easily return. Test-feeding is carried out under observation in the laboratory. During periods of low humidity a square of damp filter paper is placed beneath the cover, but this is unnecessary unless a feeding period of more than 24 hr. is required. The beans are completely disinfested by soaking in nicotine solution and are then planted 4 in. apart in seed-trays in an insect-free greenhouse. Symptoms usually appear in 17 to 25 days as the first leaves mature, but occasionally on the second flush (40 to 50 days). No plant infected in the bean has ever shown its first symptoms of virus 1 A on subsequent flushes, so that after the second flush symptomless seedlings can be regarded with certainty as not infected with this virus and the duration of experiments is shortened accordingly. The saving of greenhouse space is immense, since not only are the plants smaller and experiments completed more quickly, but the stock of virus-free seedlings essential for other techniques is unnecessary. The virus is not seed-transmitted, and ripe beans are available in sufficient quantity throughout the year.

This technique has increased the proportion of transmissions; as shown in Table 7, 100% infection in the test plants is not uncommon, with a mean of 55% transmission considering all infection-feeding sites—leaf, stem and pod.

Field transmissions

The successful use of beans for tests in the laboratory led to their use for testing the infectivity of insects found in natural outbreaks of cacao viruses, a convenient method of investigating the vectors of newly discovered cacao viruses and the virus content of potential alternative host plants.

The dissected beans are carried in specimen tubes (1 in. diameter) containing a small quantity of damp sterilized sand and sealed with muslin. Germination

* In view of the present difficulty in obtaining scientific glassware it seems worth recording that satisfactory wooden substitutes have been made locally.

commences at once, but the seedlings grow slowly under these conditions and can be held in tubes 2 in. long for at least 2 weeks before transplanting is necessary. Insects can be transferred at any stage; if germination has proceeded for more than 4 or 5 days, however, symptoms will be delayed until the second or third flush of leaves.

TABLE 7. *Bean-feeding experiments. Extracted results of various experiments in which cacao beans were used for tests*

	Source of virus	Infection-feeding time (days)	Number of test beans	Number of transmissions
Five insects per bean	Leaf	6	10	5
	Leaf*	6	4	4
	Stem*	6	16	8
	Stem	20†	5	5
	Stem + leaf	20	15	12
	Stem + leaf	20	6	4
	Pod	20	20	10
	Pod	20	10	3
	Pod	5	6	3
Ten insects per bean	Leaf*	2	17	12
	Stem*	2	16	4
	Leaf	3	10	4
	Leaf	20†	5	5
	Leaf	20	8	8
	Pod	20	8	6
	Cotyledon	20	12	6

* Leaf and stem of the same plant. † Insects bred on the source plant.

6. EXPERIMENTS WITH *FERRISIA VIRGATA*

The experiments described above were carried out with *Pseudococcus njalensis*, which is almost certainly the chief natural vector of the cacao virus 1 A in the Gold Coast. The following experiments were conducted with *Ferrisia virgata* in order to see whether this species behaved as a vector in a similar manner to *Pseudococcus njalensis*.

Infection-feeding time

Two feeding periods were used—4 and 48 hr.—on the flush leaves of the source plants, with a 3 weeks' feeding period on the test seedlings (five to ten insects per plant). Establishment was satisfactory on twenty-seven out of the thirty 48 hr. test plants, and on twenty-nine out of the thirty 4 hr. plants. Six transmissions were obtained in the 48 hr. series, and one in the 4 hr. series.

Test-feeding time

An exact duplicate of the experiment with *Ps. njalensis* outlined in § 5 was carried out with *Ferrisia virgata*. Adults and nymphs only were used, each test plant receiving between five and seven virus-fed insects. No transmissions occurred in the second series of test plants, indicating that there is no persistence of the virus in the vector. Five transmissions occurred in the first series of test plants, of which three were in the 6 hr. group, and two in the 48 hr. group.

Insect instars

Adults, third-instar nymphs, and crawlers were fed on the source plants for 48 hr. and transferred to test plants—twenty plants for each instar. Establishment was satisfactory on all of the 'adult' and 'nymph' plants, but on only sixteen of the 'crawler' plants. The infestation rate was seven crawlers, five adults or four nymphs per plant. Four crawler, two nymph, and one adult infested plants became infected, indicating that, as with *Pseudococcus njalensis*, the crawlers are the more efficient vectors.

Bean feeding

All instars of *Ferrisia virgata* feed readily on cacao beans. In experiments in which five nymphs were transferred from the leaves of infected plants to each bean, 80% transmission was obtained when the insects had been reared on the source plant and fed for 24 hr. on the beans.

Conclusions

Some experiments described in this paper are empirical while others are inconclusive. It should be remembered, however, that beyond the fact that the mealybugs, *Pseudococcus njalensis* and *Ferrisia virgata*, could transmit the swollen-shoot virus 1A nothing definite was known about the conditions of transmission. The facts and experience gained in these experiments formed the basis for more intensive investigations into feeding times, effect of starvation and the vectors of other cacao viruses, investigations which have contributed to the perfection of technique but which will appear more appropriately in a paper on virus-vector relationships. It may be mentioned that later investigations have confirmed the conclusions which have been drawn from the experiments here described and which may be summarized as follows:

(1) All stages of *Pseudococcus njalensis* and *Ferrisia virgata* can transmit cacao virus 1A, nymphs being more efficient vectors than adults.

(2) The vectors can become infective after less than 4 hr. feeding.

(3) The vectors can infect a plant in less than 3 hr. feeding; the virus is non-persistent in the insect.

(4) Young leaves ('flush') showing symptoms provide the best source of virus for the infection-feed, although the vectors can also obtain virus from stem or pod.

(5) The latent period in seedlings is very variable, leaf symptoms being dependent on new growth-flushes; when cacao beans are used for tests this variation is greatly reduced. From all considerations, including economy of space, control of vectors and percentage of transmission, beans are the most satisfactory test stage for use with mealybugs.

This paper is published by kind permission of Mr O. J. Voelcker, Director of Cacao Research.

REFERENCES

Box, H. E. (1945). Insect transmission of the swollen-shoot virus. *Nature, Lond.*, **155**, 608.
Carter, W. (1945). The oral secretions of the pineapple mealybug. *J. econ. Ent.* **38**, 335.
Cotterell, G. S. (1943). Entomology section, *Rept. on Central Cocoa Res. Sta. Tafo*, 1938–42. (Ed. M. Greenwood.) Accra: Gold Coast Printing Dept.
Elmer, O. H. (1925). Transmissibility and pathological effects of the mosaic disease. *Iowa agric. Exp. Sta. Res. Bull.* no. **82**, 38.
Olitsky, P. K. (1925). The transfer of tobacco and tomato mosaic diseases by *Pseudococcus citri*. *Science*, **62**, 442.
Posnette, A. F. (1941). Swollen-shoot virus disease of cacao. *Trop. Agriculture, Trin.*, **18**, 87.
Posnette, A. F. (1947*a*). Virus diseases of cacao in West Africa. I. Cacao viruses 1A, 1B, 1C and 1D. *Ann. appl. Biol.* **34**, 388.
Posnette, A. F. (1947*b*). The use of seeds in the insect transmission of some plant viruses. *Nature, Lond.*, **159**, 500.

(*Received* 1 *July* 1947)

OBSERVATIONS ON THE DEVELOPMENT OF THE COTTON BOLL, WITH PARTICULAR REFERENCE TO CHANGES IN SUSCEPTIBILITY TO PESTS AND DISEASES

By R. C. RAINEY

Empire Cotton Growing Corporation, Barberton, Transvaal

(With 4 Text-figures)

Continuous and well-marked changes in composition are demonstrated during the development of the cotton boll, and discussed in relation to changes in susceptibility to pests and diseases occurring over this period.

Cotton buds and flowers, the main food of the earlier instars of *Heliothis* and *Diparopsis*, and, at the beginning of the season, of *Platyedra*, represent the richest recorded source of nitrogen available to the larvae.

The first 2 weeks of boll development, when most physiological shedding occurs, are characterized by extremely rapid growth, the dry weight of the ovules being approximately doubled every 2 days throughout this period.

The developing ovules are richest in reducing sugars during the second and third weeks, when moisture-content is also highest. Bolls of this age are attacked by the later instars of *Heliothis* and *Diparopsis*, which are the stages at which the accumulation of larval fat is likely to be most active. Male *Dysdercus*, which can survive for prolonged periods on simple sugar solutions, show a marked preference for bolls of this age, which are punctured to a varying extent by other stages of *Dysdercus*, probably primarily as a source of water. This is also the stage at which attack by *Nematospora* is most damaging; reducing sugars provide a highly suitable source of carbon for this fungus.

The cellulose of the mature lint and the oil and protein of the ripe seed are mainly laid down after the boll reaches full size (at 4–5 weeks under local conditions), about half-way through its maturation period, and are largely derived from materials entering the boll in the course of its subsequent development. Premature senescence, such as that associated with *Alternaria* attack, can thus affect yield even when defoliation does not occur until after most of the bolls have attained full size.

The ripening seed, becoming steadily richer in oil and protein during the latter part of boll development, forms the main food of *Platyedra* towards the end of the season, a change of diet reported to induce the larval diapause. Ripe seed provides food which is essential for satisfactory nymphal development and probably also for oviposition in *Dysdercus*; specific protein requirements are possibly involved.

Examples of direct effects of environmental factors on the development and composition of the boll are described. Over the range of conditions experienced by the experimental material these effects were relatively small.

Introduction

In the course of studies on translocation in the cotton plant, Maskell & Mason (1930) demonstrated a threefold increase in the concentration of reducing sugars, with a fall in protein of similar extent, in the sap of the developing ovules during the first week of boll development. Phytophagous insects have been found to be markedly

affected by changes of this nature in the composition of the host-plant (Evans, 1938, etc.); and the distribution of a number of insects on different stored products has since been shown to be closely associated with differences in basic food requirements (Fraenkel & Blewett, 1943). An investigation of the changes in various chemical constituents during the whole period of boll development was accordingly undertaken at Barberton during 1939–40, in the hope of throwing some light on the marked changes in susceptibility to pest attack which have been observed to occur in the course of boll development.

Material

The variety used in most of the work was the Upland strain U. 4/4–052, which was in general cultivation in the South African Low Veld during 1935–9. Supplementary data were obtained in 1940 from strain 5143, a derivative of 052 which replaced it in general cultivation in that season. The 1939 material was obtained from two fields of 052 on the diorite loam soil of the Cotton Experiment Station. The first was planted on 22. x. 1938 in a field of comparatively low fertility, and, despite a 200 lb./acre dressing of superphosphate, the crop gave only 450 lb. seed-cotton per acre. The second field, planted on 7. xii. 1938, received per acre 7 tons compost, 200 lb. sulphate of potash and 200 lb. superphosphate, and gave 810 lb. seed-cotton per acre, which is considered good for a late planting.

In both plantings, material was obtained from dated flowers opening 2–3 weeks after the start of flowering, bagged 2 days after anthesis to avoid insect attack. Duplicate samples of the resulting bolls were withdrawn at weekly intervals and subdivided to provide for parallel determinations of sugars, carried out on fresh material, and of moisture-content, giving dried material for the estimation of other constituents. Diseased and malformed bolls were rejected. Representative bolls from each sample were pickled in formal-acet-alcohol for subsequent morphological examination.

The bagged bolls remaining on the plants after the periodic withdrawals were collected at maturity, and the commercial characteristics of the resulting seed-cotton were recorded.

Samples of buds at two stages, and of ovaries, from fresh flowers, were also taken. Detailed work was restricted to the developing ovules, which were separated from the 'carpels'—boll-wall, septa, etc.—at all stages from the flower to the ripe open boll.

The 5143 material consisted of healthy unbagged bolls, of known age and position, collected during January–March 1940, from a field on granite soil planted on 24. x. 1939, in the course of a plant pathological survey by Dr G. M. Wickens.

Analytical methods

Moisture. Weighed samples were dried to constant weight (within $\frac{1}{2}$%) in a steam-oven.

Sugars. Ovules were removed from duplicate samples of 4–20 bolls each, plunged into boiling water, ground to a smooth paste with sand, and extracted with further

hot water. The extract was cleared with saturated lead acetate, and excess of the latter removed with solid sodium oxalate. Reducing sugars were determined in the resulting solution by the modified Hagedorn-Jensen-Hanes method (Hulme & Narain, 1931). Oligosaccharides were similarly determined after hydrolysis by an invertase-melibiase preparation; the method used was found to give quantitative recovery of sucrose and raffinose added to fresh material before extraction. An attempt to estimate these two sugars separately was unsuccessful, commercial invertase and local samples of top and bottom yeasts failing to give preparations differing sufficiently in melibiase activity for this purpose.

Nitrogen. Total nitrogen was determined by the Kjeldahl and micro-Kjeldahl (Parnas-Wagner) methods. The results of Maskell & Mason (1930) and Dastur & Ahad (1945) indicate that 70–90% of the total nitrogen of the developing ovules is in the form of protein, and that most of the remaining nitrogen is in the form of amino-acids and amides likely to be equivalent to protein in nutritive value. For the preliminary consideration of the nutrition of organisms attacking the boll, total nitrogen may therefore usefully be expressed as crude protein, as is often similarly justifiable in nutrition studies on forage crops (Chibnall, 1939).

Oil. Dried material was crushed in a mortar and continuously extracted for 36 hr. with ether to constant weight.

Cellulose was determined by a modified Cross and Bevan method (Dorée, 1933) in lint samples from bolls 6 weeks of age and onwards, from which dried material was still available when work was resumed in 1946. The oven-drying to which these samples had been subjected was found to be without effect on the yield of cellulose.

Significant differences. All determinations were carried out on duplicate samples, and the differences between occasions, plantings, etc., necessary for significance at the 5 and 1% level, were computed. Sampling variances were estimated by pooling all comparable data, within the limits imposed for example by the rapid early growth of the boll.

I. Changes in the principal chemical constituents of the developing boll

(*a*) *The course of boll development in a typical series*

The growth of the developing ovules and carpels in fresh and dry weight is shown in Fig. 1 for the boll samples of series II (*vide infra*), from the later 1938–9 crop.

The growth rate of the ovules was extremely high during the earlier part of boll development, dry weight being doubled on the average every 2¼ days during the first 2 weeks. Growth at this rate might be expected to be very vulnerable to temporary interruptions in the supply of nutrients, and it is in fact during this period that the boll is particularly liable to physiological shedding—which, even in a good crop, may account for more than half the total bolls set. Thus at Barberton, MacDonald, Fielding & Ruston (1941 *et seq.*) showed that boll shedding, other than

that due to insect damage, occurred mainly 7–10 days after flowering, and similar observations have been recorded in the Belgian Congo (Soyer, 1937), India (Joshi, Gode & Shah, 1941), and Nyasaland (Pearson & Mitchell, 1945).

Between 4 and 5 weeks the growth of the boll-wall ceased, with no further increase in the fresh or dry weight of the carpels, or in boll diameter (Fig. 2), and the fresh weight of the developing ovules attained its final value at the same time.

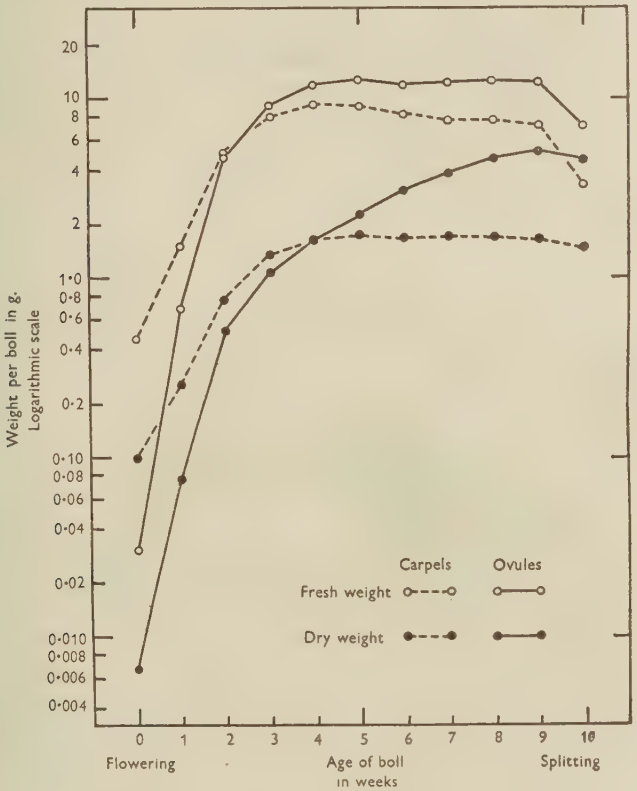


Fig. 1. Changes in fresh and dry weight during boll development (series II).

The dry weight of the ovules, however, continued to rise steadily, with highly significant weekly increases up to the age of 9 weeks. Balls (1915), working with Egyptian cotton, first showed that the developing boll grows to its full external dimensions in the first half of its maturation period, and similar results were subsequently reported for Upland strains in Texas (Martin, Ballard & Simpson, 1923) and in the Congo (Soyer, 1937).

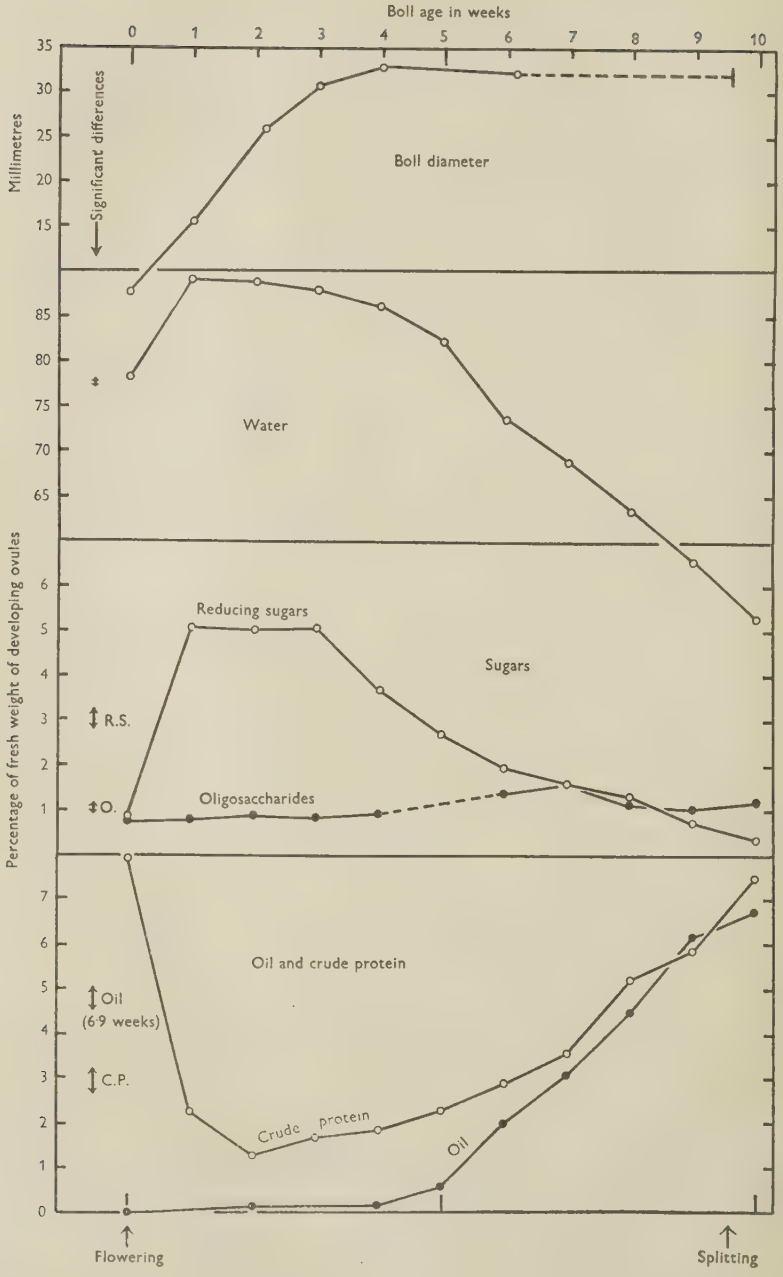


Fig. 2. Changes in the composition of the developing cotton boll (series II).

During the period of growth in size the ovules were rich in reducing sugars (approaching 50%, dry weight at 1–2 weeks), with a high moisture-content (85–90%), but contained very little oil and relatively little protein (Fig. 2). Balls showed that much structural differentiation occurs after the boll attains full size—hardening of the seed-coats, differentiation of the embryo, and thickening of the lint—and Table 1 shows that during this period the preliminary scaffolding of

TABLE 1. *The development of the cotton boll*

Variety 052, planted 7. xii. 1938; 1500 flowers tagged 1. iii. 1939, and samples withdrawn at weekly intervals.

	Weights per boll of constituents of developing ovules					
Boll age (weeks)	Water (g.)	Reducing sugars (mg.)	Oligo-saccharides (mg.)	Crude protein (mg.)	Oil (mg.)	Lint cellulose (g.)
0 (flowering)	0·025	0·27	0·23	2·5	0·009	—
1	0·614	35	5·4	15·6	—	—
2	4·17	236	40	59	6·5	—
3	8·05	463	75	151	—	—
4	10·13	433	107	215	16	—
5	10·41	339	—	286	77	—
6	8·67	229	161	343	234	0·89*
7	8·47	196	193	437	379	1·08
8	7·92	163	142	649	559	1·42
9	6·99	88	122	704	745	1·64
10 (just split)	2–5	33	114	713	640	1·51
Sig. diff. (P=0·05), with period in weeks for which applicable	0·59 (2–9)	48 (2–8)	37 (3–9)	80 (4–9)	56 (6–9)	0·12 (6–10)

* Earlier material not available.

sugars and water was almost completely replaced by the constituents of the mature boll-protein, oil and cellulose. Thus 88% of the oil and 60% of the nitrogen of the mature seed-cotton were laid down after 5 weeks, when growth in size had ceased. Similarly, over 40% of the lint cellulose was formed after 6 weeks, although the lint had already reached its full length by this stage. The potential adverse effect of factors such as the premature senescence associated with *Alternaria*, even when defoliation does not occur until after most of the bolls have attained full size, is thus emphasized. A significant negative correlation between total yield and senescence grade, as assessed some 10 weeks after the beginning of flowering, has in fact been demonstrated, both between and within strains (MacDonald *et al.* 1946).

Table 2 shows that the percentage of cellulose in the developing lint continued to rise steadily up to the time of boll opening, with significant increases between each pair of successive weekly samples. (This observation suggested that cellulose content might incidentally afford a useful measure of the maturity of lint samples, but lint

showing the characteristic silvery appearance of marked immaturity (series I*b*) gave a cellulose-content of 92·9% dry weight, which just failed to differ significantly from the value of 93·7% given by normal lint (series II).)

TABLE 2. *Changes in cellulose in the developing lint*

Material as Table 1.

Boll age (weeks)	Cellulose content of lint dry weight (%)
6	70·9
7	74·3
8	80·0
9	85·7
10	91·9
(just split)	
Picked normally	93·7

Significant differences ($P=0{\cdot}05$): (i) 6–9 weeks, 3·2%; (ii) split and picked, 0·9%.

Reducing sugars were at a maximum during the early phase of rapid growth, and not, as might perhaps have been expected, when the formation of cellulose, oil and protein was most active, which was about a month later. However, the total dry weight of the contents of the 4-week-old boll, in which reducing sugars were just beginning to decline, corresponded to little more than a third of the dry matter of the final seed-cotton, most of which must therefore have entered the boll in the course of its subsequent development. The materials of the young boll can accordingly have provided comparatively little of the precursors of the constituents of the mature seed-cotton. Thus between 4 weeks and maturity each boll laid down 620 mg. of oil, 500 mg. of crude protein, and about 1000 mg. of lint cellulose, with a corresponding decline in reducing sugars of only 400 mg. Caskey & Gallup (1931), reporting a similar decrease in reducing sugars with rising oil content in a study of the latter part of boll development in Upland cotton in Oklahoma, also pointed out that their figures in themselves did not provide convincing evidence of the formation of oil at the expense of sugars. Dastur & Ahad (1945) regarded the general decline in the proportion of reducing sugars during boll development as evidence that these sugars were probably the main carbohydrate utilized in the formation of protein, oil and cellulose, but presented only results expressed as percentages of dry matter, without corresponding observations of actual weights per boll. Furthermore, of course, the precursors of the constituents of the mature boll need not attain high concentrations at any stage if synthesis keeps pace with translocation.

Oligosaccharides attained higher concentrations after growth in size had ceased, but interpretation of these observations is complicated by the differing physiological roles of the sugars included in this class. Sucrose has been suggested as the main form in which sugars enter the very young boll (Mason & Maskell, 1928) while raffinose is probably primarily a reserve material, up to 8% occurring in cotton-seed meal.

Table 1 also gives an indication of the demands made on the plant by the developing boll, underestimated of course by the amount of any constituent lost by respiration, etc. Thus the net water uptake rate of the boll was at a maximum at 1–4 weeks. The weight of water per boll was highest at 5 weeks, and amounted to about twice the weight of the seed-cotton finally produced. The demand for assimilates, as estimated by weekly increments of dry weight, varied less with age than the demand for water, but tended to be highest at 5–7 weeks. Nitrogen was also taken up continuously throughout boll development, with a maximum rate of uptake between 6 and 8 weeks. The fruiting requirements of the crop thus demanded a continuous supply of nitrogen over a period of at least 3 months.

To sum up, the development of the cotton boll involves well-marked changes in a number of its major chemical constituents. Two main phases of boll development, of roughly equal duration, may be distinguished. The first is characterized by rapid growth in size, high water-content, and abundance of reducing sugars, while during the second phase most of the cellulose, oil and protein of the mature seed-cotton are laid down, with no further growth in the external dimensions of the boll.

(*b*) *Effects of variations in growing conditions on the course of boll development*

Before proceeding to discuss variations in susceptibility to pest attack, it is desirable to consider such evidence as is available to show how far the foregoing results, given by the bolls produced by the flowers of a single day in a single crop, may be modified by variations in growing conditions, and to indicate the range of material to which these results are likely to apply.

(i) *Range of observations available*

The variations in growing conditions experienced by the experimental material may first be summarized.

Series I was derived from the flowers of 16. i. 1939, the 86th day after planting, in the earlier 052 crop on the poorer loam soil. A few observations are also available from material provided by the flowers of 19 January (series I*a*), 23 (I*b*) and 26 (I*c*) in the same crop. The final yield was relatively low. Very heavy rains—including one overnight total of more than 9 in.—occurred over a short period in early February, during the early development of these bolls, and observations on a similar adjoining crop showed that practically all bolls formed subsequent to the rains were shed, and that no further buds were produced (MacDonald *et al.* 1940).

Series II was derived from the flowers of 1. iii. 1939, the 84th day after planting, in the later 052 crop on the more fertile loam soil, which gave a satisfactory yield.

Series III and IV were provided by the following year's crop of 5143 on the granite soil, which promised well at the beginning of the season but subsequently closed down early. The two series were derived respectively from flowers produced 85–7 and 102–5 days after planting, representing the earliest and latest bolls of which adequate numbers were available on all sampling occasions, and therefore differed considerably in their position on the plant. Thus 93% of the bolls comprising series III were borne on the sympodia of the main axis, and only 7% on those of the basal monopodia, while the corresponding proportions for series IV were 62 and 38%.

Differences in the weather experienced by the four series are illustrated by ranges of 2·5–14·4 in. for total rainfall, 198–296 hr. for total sunshine, 81–86° F. for mean daily maximum temperature, and 61–66° F. for mean daily minimum, over the first 6 weeks of boll development.

It has been shown (e.g. MacDonald *et al.* 1941) that the bulk of the Low Veld crop is normally produced by bolls set during the first month or so of flowering, about 70–110 days after planting—though the destruction of most of these bolls by American bollworm can result in the production of a full crop by the later bolls, which are normally shed (Parsons & Marshall, 1941). The four series thus refer to bolls, bagged and unbagged, reasonably representative in time of formation of the

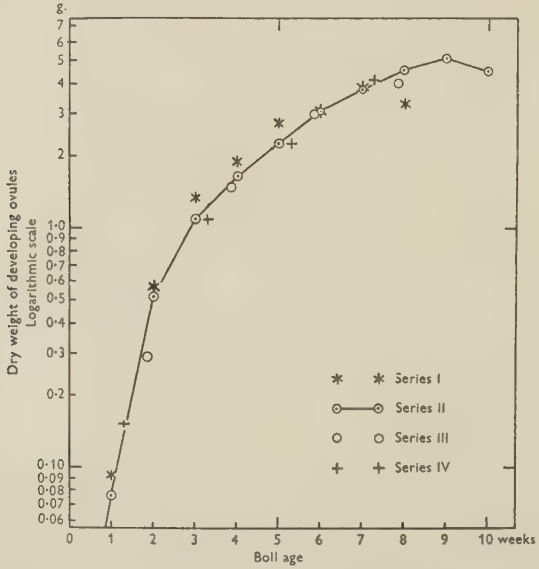


Fig. 3. Boll growth rates under varying conditions.

bulk of each crop, developing over four different periods during two seasons in three fields of varying fertility level on two types of soil, and include both of the varieties commercially grown in the Low Veld in recent years.

(ii) *Dry-weight data*

While the data are insufficient to enable the effects of each of the factors just mentioned to be tested separately, the combined effects on boll-growth rate, as indicated by differences between series in the dry weight of the ovules, are not large (Fig. 3). At 3, 4 and 5 weeks series I attained values equivalent to those of bolls 3–4 days older in series II, a difference which was fully significant on the latter occasion. By 6 weeks, however, all four series gave dry weights agreeing well

within the limits of their respective sampling errors (though there were in fact differences in particular constituents—*vide* Table 4). This agreement in dry weight can scarcely be attributed to the approach of maturity, and hence of a limiting value of boll weight, since growth was still actively in progress at 6 weeks; the increase in dry weight between 6 and 7 weeks was highly significant in both series I and II. Series I, despite good initial growth, gave a lower weight at maturity than the other series, suggesting that adverse conditions were taking effect during the latter part of boll development. There was no systematic difference between series III and IV, despite their differing positions on the plant, nor between these and series II of the previous season.

TABLE 3. *Rainfall and the moisture-content of 2–3-week-old bolls*

	Date sampled	Boll age in days	Moisture-content of Carpels (%)	Moisture-content of Ovules (%)	Rainfall between sampling dates (in.)
Series I	30. i	14	83·87	88·28	11·15
	6. ii	21	83·76	86·64	
Decrease in moisture-content			0·11	1·64	
Sig. diff. (P=0·05)			0·76	1·33	
Series II	15. iii	14	84·97	88·98	0·13
	22. iii	21	83·21	87·99	
Decrease in moisture-content			1·76	0·99	
Sig. diff. (P=0·05)			1·05	0·58	

(iii) *Moisture-content data*

Moisture content determinations on the ovules of series I and II showed very similar trends, with a steady fall after the age of 1–2 weeks. The first series, despite wetter conditions, was consistently ahead of the second in this respect, by an amount equivalent to 5–7 days' development (Rainey, 1940).

Even the immediate effects of the rains on the moisture-content of the developing bolls were comparatively slight (Table 3). There was a fully significant decrease (P=0·02) in the moisture-content of the developing ovules in series I during the week of the rains, which even exceeded the corresponding decrease in series II. The decrease in the moisture-content of the carpels over the corresponding period was, however, significantly greater in series II than in series I, which may be regarded as a direct effect of rainfall on the composition of the boll wall. This may have been a factor in the development of a characteristic malformation, with incomplete union of the carpels at the tip providing easy access for boll-rotting bacteria, which was exhibited by a number of the bolls of series I. This malformation, which has also been recorded from Rhodesia (Hopkins, 1932), was observed during this season only in bolls which had experienced these rains during their early development (Wickens, 1940); it may be alternatively attributed to failure of the corolla to shed after anthesis under wet conditions (Pearson, unpublished communication).

The moisture-content of the carpels showed in general rather less agreement

between series than that of the ovules, and provided further indications of direct effects of rainfall on the moisture-content of the boll-wall. Such effects may also have some bearing on the direct penetration of the boll-wall by *Xanthomonas malvacearum* (E.F.Sm.) Dowson in the development of bacterial boll disease.

(iv) *Sugar-content data*

The changes in reducing sugars during boll development in the earlier crop, like those in moisture-content, showed general agreement with those observed in series II. High concentrations, around 6%, were recorded in both at 1–3 weeks, and a steady fall thereafter, with the earlier series (I*c*) about a week ahead in this respect at 4 and 5 weeks, and about 2 weeks ahead from 6 weeks onwards (Rainey, 1940).

The heavy February rains, while without demonstrable effect on the moisture-content of the developing ovules, were associated with a depression of reducing sugar-content, in which both waterlogging and reduced insolation may have been concerned. Thus ovules of series I*a*, sampled at 2 weeks of age immediately before the heavy rains, gave a reducing sugar-content of 6·1%, while 2-week-old bolls of series I*c*, sampled just after these rains in the same crop, contained only 4·8%, the difference of 1·3% being significant. Series I*a* had experienced 0·13 in. rain and 43 hr. sunshine during the second week of its development, while the corresponding figures for series I*c* were 11·95 in. and 17½ hr. Considering these differences in weather conditions, the observed depression of the sugar-content was not large, and Steyaert (1938), comparing reducing sugars in 16-day-old bolls from eight different sowing dates in the Congo, found only a single significant difference, with no discernible correlation with climatic factors.

TABLE 4. *Sugars, oil and water in 6-week-old bolls of early and late plantings*, 1938–9

Mean weights per boll of constituents of developing ovules at 42 days from flowering.

	Early planting (series I and I*c*)	Late planting (series II)	Difference between plantings	Significant difference ($P=0.05$)
Water	7·06 g.*	8·67 g.	1·61 g.	0·93 g.
Dry weight	3·06 g.*	3·10 g.	0·04 g.	0·29 g.
Reducing sugars	143 mg.†	229 mg.	90 mg.	35 mg.
Oligosaccharides	89 mg.†	161 mg.	75 mg.	29 mg.
Oil	371 mg.*	234 mg.	137 mg.	56 mg.

* Series I. † Series I*c*.

(v) *Maturation rates*

As already mentioned, although all four series attained the same dry weight at 6 weeks, there had been differences in their earlier history, and Table 4 shows that there were in fact significant differences in sugars, oil and water between 6-week-old samples from the two 1938–9 crops. As a further illustration of the extent to which the moisture-content of the ovules appeared to be independent of the rainfall received during boll development, series II contained 23% more water than

series I at this stage, though the first series had received nearly six times as much rain since anthesis. The earlier material had experienced mean temperatures $2\frac{1}{2}$–5° F. higher, and its higher oil-content suggests that it was physiologically older at this stage. The differences in sugars and water may in part be interpreted similarly, but adverse growing conditions may also have contributed to lower sugar- and water-contents. Making some allowance for such an effect, the difference in physiological age between the two series at 6 weeks may be estimated, from a comparison of Tables 1 and 4, as about a week.

The mean maturation periods finally observed for series I and II were respectively 55 and 67 days. Maturation periods of this order are typical of Barberton conditions; the considerably shorter periods recorded for Upland strains in the United States, such as 43–5 days in Texas (Martin *et al.* 1923) may perhaps be attributed largely to higher temperatures. An average summer temperature of 77° is said to mark the northern limit of the U.S. Cotton Belt (Brown, 1938), while the mean temperature of the hottest month of the year at Barberton (January) is only 73·9°. Incidentally, mean temperatures in the cotton-growing areas of Uganda similarly lie between 70° and 72°, with a variation from July to January of about 4° (Walter, 1940).

(vi) *Properties of the seed-cotton produced by the experimental material*

Table 5 summarizes the commercial characteristics of the seed-cotton produced by the 1938–9 experimental material. Corresponding mean values for 052, from the five variety trials in which it was grown on the local loam soil during this season, are included for comparison, to indicate the extent to which the experimental material was representative of the crop as a whole.

TABLE 5. *Commercial characteristics of experimental material* 1938–9

	Yield (lb. lint/ acre)	Lint length (mm.)	Lint index (g./100 seeds)	Seed-weight (g./100 seeds)	Ginning percentage	Seed-cotton/ boll (g.)	Seeds/ boll
Early planting	170	—	—	—	—	—	—
Series I	—	29·5	5·1	8·4	37·7	4·2	31
Series I*b*	—	30·4	4·4	7·5	37·3	3·8	32
Late planting	270	—	—	—	—	—	—
Series II	—	31·7	5·1	10·1	33·6	5·1	34
Standard 052 data							
Mean values from five variety trials planted 18–30. x. 1938	237	28·9	4·5*	8·4*	34·8*	—	—

* Relate to first pickings only.

Series II was somewhat above average in seed-weight, lint index and lint length, and slightly below average in ginning percentage. Consistently higher lint indices and seed-weights were given by the first pickings (representing the bolls derived from about the first month of flowering), as compared with later pickings, in all the

variety trials. Series II, which originated about a fortnight after the start of flowering, may therefore have been somewhat better than average for the crop in which it was produced.

The decline in lint index and seed-weight in later bolls was well illustrated in the earlier crop, series I*b* giving a lint index and seed-weight both significantly lower than series I, derived from the flowers of a week earlier. The low sugar-contents recorded earlier for series I*c* may have been associated with this effect. Despite the low lint index of series I*b*, lint length was still above average, indicating that the effects of adverse conditions had made themselves felt relatively late in boll development (Balls, 1915), as was also suggested by the dry-weight data. This lint was classed by the plant-breeding staff before ginning as immature and lacking in body.

Series I gave significantly less seed-cotton per boll than series II, in agreement with the growth-rate data. To this difference lower seed-weight and lower number of seeds per boll both contributed significantly, but there was on the other hand no difference in lint index between these two series. The less well-filled seed of series I—and the poor showing of the following week's material—were probably associated with the early closing down of this crop, though, despite the poor final yield, the lint length, lint index and seed-weight of series I were all well up to the average for the season.

It is therefore concluded that, over the range of conditions covered by this material, the direct effects of environmental factors on boll development were small compared with their effects on such processes as bud- and boll-shedding, and that their effects on boll composition were small compared with those of boll age. Hutchinson (1940) has pointed out that the effects of environmental fluctuations on the number of bolls per plant are much greater than on the weight of seed-cotton per boll, and also showed (1943) that in St Vincent material the hair characters and spinning value of cotton lint were remarkably little affected by the more easily measured environmental influences.

II. Variations in susceptibility to pests and diseases during boll development

In view of the observations just recorded, it is concluded that the results described for series II may be regarded as of reasonably general application. These developmental changes in boll composition may accordingly be discussed in relation to the nutrition of some of the pests concerned.

(*a*) *Internal boll disease* (Nematospora)

Pearson (1934, 1939, 1947), studying the effects of artificial inoculation with *Nematospora*, noted a marked decrease in susceptibility to staining with boll age, heavy discoloration occurring only in bolls inoculated before the age of 4–5 weeks. Wickens (1947), using stainers carrying *Nematospora* as well as an improved technique of artificial inoculation, found severe rotting of the boll contents in material

inoculated before the age of 3–4 weeks, while bolls inoculated at a later stage showed little if any rotting, with lint staining, heavy at 4 weeks, progressively reduced in intensity with advancing age at the time of inoculation.

The possibly suggestive parallel between the striking decline in reducing sugars found in the present investigation (from nearly 50% of total dry matter at 1 week to less than 1% in the mature boll) and the changes in reaction to *Nematospora* over the same period, has already been briefly noted (Rainey, 1940). Farries & Bell (1930) found that fructose, glucose and mannose, particularly the first, were very favourable sources of carbon for *Nematospora*.

Steyaert (1938) independently drew attention to the possible importance of boll sugars in relation to susceptibility to *Nematospora*. He demonstrated significant differences in the reducing sugar content of 23-day-old bolls between a number of Upland strains in the Congo, together with significant differences between these strains in susceptibility to *Nematospora*, as assessed by the size of lesions produced by standard inocula in cut bolls. These preliminary results gave no correlation between sugars and susceptibility, but M. Steyaert has agreed that further work, particularly on the assessment of susceptibility, is necessary before a final pronouncement can be made on this point. The inoculations (Steyaert, 1939) were carried out on cut bolls kept in sterile water from the 16th to the 25th day after flowering, and consideration of the normal growth rate at this stage suggests that such cut bolls are unlikely to remain long in a physiologically normal condition. Thus the present data gave an increase of 124% in the dry weight of the boll contents over this period, and absence of nutrients to this extent might be expected to have a somewhat drastic effect on the final condition of the material. It is furthermore doubtful, in view of the marked variation in both sugars and susceptibility with boll age, whether estimates of both on bolls of a single age provide sufficient material for adequate comparisons between varieties. Differences in physiological age between bolls of the same chronological age may well give appreciable differences in both sugars and susceptibility; for example, the present material, at the same age as Steyaert's, required only 3 days' growth to give a fall in reducing sugars equal to the difference (0·62% fresh weight) between his highest and lowest varieties. The interpretation of some recent work in Peru on varietal resistance to internal boll rot due to *Dysdercus* (Boza Barducci, Garcia Rada & Wille, 1945), using cut bolls inoculated by a similar method with *Alternaria* sp., *Acremonium* sp., and an unidentified bacterium and fungus (though not *Nematospora*), is subject to similar objections.

The existence of useful differences between varieties in susceptibility to *Nematospora* thus has still to be satisfactorily demonstrated, and the relationship between sugars and susceptibility likewise remains an open question. Should further work establish such a relationship, a low reducing sugar-content during early boll development may of course prove incompatible with a high yield of cellulose, though the relatively minor part which the sugars present in the young boll can play quantitatively as precursors of the cellulose—particularly the secondary thickening—

of the mature lint has already been indicated. Furthermore, Pearson (1934, 1947) has pointed out that the observed decrease in susceptibility to staining with advancing boll age may be alternatively explained, at least to some extent, in terms of the increasing vacuolation of the lint hairs, with the characteristic discoloration taking place in a steadily diminishing amount of protoplasm.

(*b*) *Stainers* (Dysdercus)

Observations by Pearson (1934, 1937) on the diet of nymphs of *D. nigrofasciatus* Stål (Pyrrhocoridae) may first be summarized as follows:

> There is no feeding during the 1st instar; in the 2nd instar difficulty is experienced in penetrating older bolls, though stylet length already exceeds boll-wall thickness. 2nd and 3rd instar nymphs feed in the field on green bolls of up to 3 weeks of age, and must also have access to full-grown split bolls for satisfactory development.
>
> 4th and 5th instar nymphs feed on bolls of all ages, but to a much greater extent on those between 2 and 6 weeks old in the field.
>
> At high humidities a strong preference was shown for older bolls, and puncturing in general was greatly reduced. '...progressive decreases in mortality, and in length of stadium of those which moulted, were observed on a series of bolls of increasing size...split open to eliminate any possibility of mortality due to inability to penetrate the boll wall.'
>
> Pearson concluded that 'the extent to which different stages of *D. nigrofasciatus* feed on bolls of different ages...under field conditions...is apparently conditioned partly by desire for moisture and partly by desire for nutrition.'

Considering the foregoing in relation to Fig. 2, it may be suggested that feeding on bolls of up to 3 weeks of age is primarily a quest for water, while the progressively better growth obtained on older bolls, and the need for access to split fruit, may perhaps be associated with the low protein content of the younger bolls.

Bolls up to about 3 weeks of age, which thus appear to be utilized by stainers primarily as a source of water, are at the stage most susceptible to *Nematospora*, which *Dysdercus* transmits, and, in view of the observed effects of humidity on puncturing, these young bolls are likely to be attacked most heavily at low humidities. Other things being equal, dry conditions over the period when the bulk of the crop is at this stage, say 80–100 days from planting under local conditions, might therefore be expected to be conducive to heavy stainer damage. The worst stainer damage ever experienced on the Cotton Station at Barberton was in 1931, when only 0·36 in. of rain fell between 74 and 101 days after planting, much the lowest total ever recorded over this period during the 21 years that the Station has been in operation.

Data on the boll-age preferences of stainers were also provided by field caging experiments (Rainey, 1941), with results exemplified by Fig. 4. Adult males and 5th instar nymphs were used to investigate possible differences between the primarily maintenance requirements of the former and the demands of active growth in the latter. The nymphs gave results in close agreement with Pearson's field observations, while the males showed a marked preference for the younger bolls, of high moisture-content and rich in sugars though relatively poor in protein.

A simple sugar solution, corresponding approximately in composition to cotton nectar, has been shown to provide an adequate 'maintenance ration' for the adults of this species, giving in fact even lower death-rates than a diet of seed-cotton, and it is probably the nectar supplied by various flowering trees of the Low Veld which normally enables this species to overwinter (Pearson, 1939). Preliminary caging experiments with female stainers indicated a preference for bolls older than those on which the males fed most freely, which may perhaps be associated with Pearson's observation that oviposition never occurred on a nectar diet, while taking place freely on seed-cotton. The absence of breeding on food-plants outside the Malvales, such as *Acacia pallens* Rolfe (Leguminosae) and *Gymnosporia acuminata* Szysz. (Celastraceae) (Pearson, 1937), may also be interpreted as evidence of specific protein requirements for oviposition, similar for example to those of many blood-

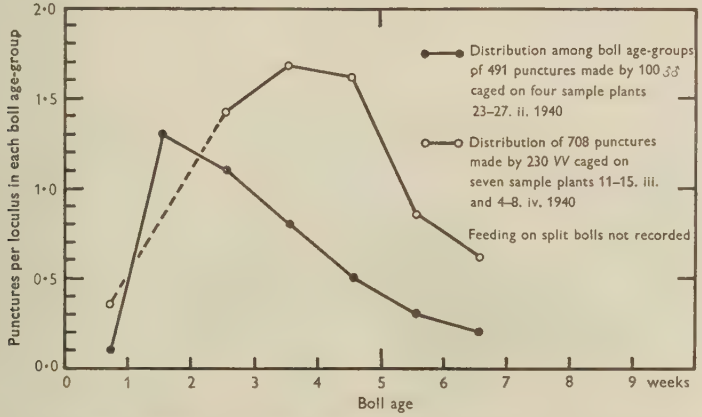


Fig. 4. Feeding preferences of *Dysdercus nigrofasciatus* Stål. Testing distribution of punctures for independence of boll age gave values of χ^2 corresponding to $P<0{\cdot}001$ for both ♂♂ and *VV*.

sucking insects. The rate of reproduction of the cabbage aphis, *Brevicoryne brassicae* L., has been shown to be positively correlated with the nitrogen-content of the host plant, and, in particular, with the protein content (Evans, 1938).

Pearson (1934) also noted that 'the characteristic proliferations associated with the puncturing only developed in bolls up to 4 weeks of age when punctured'. Fig. 1 shows that this is the stage at which growth of the boll-wall ceases; Pearson further observed that proliferations developed within 5 days, and that bolls in the stage of very rapid growth (10 days old) developed the greatest percentage of proliferated punctures.

(*c*) *Bollworms* (Heliothis, Earias, Diparopsis, Platyedra)

Table 6 summarizes some typical local field observations on the feeding-sites of different instars of the American bollworm (*Heliothis armigera* Hübn., Noctuidae).

As more than 60% of the eggs are laid on the vegetative parts of the plant (Parsons & Ullyett, 1936), larval feeding-sites bear little relation to oviposition sites, and would therefore appear to be actively selected by the larvae themselves.

TABLE 6. *Feeding preferences of larvae of* Heliothis armigera *Hübn. on cotton*

(Parsons & Marshall, 1940, unpublished)

Distribution of feeding larvae on thirty sample plants of varieties 052, 5143 and 929

	Numbers of larvae		
Feeding sites	1st and 2nd instars	3rd instar	4th, 5th and 6th instars
Leaves, small	3	7	3
Leaves, large	—	2	—
Buds	78	44	38
Bolls, 0–2 days old	29	18	18
Bolls, 3–4 days old	25	15	15
Bolls, 6–19 days old	4	10	20
Bolls, 20–44 days old	2	—	5
Bolls, over 44 days old	—	—	—

Buds and very young bolls formed practically the sole food of the younger larvae, and continued to provide much of the diet of the later instars. Table 7 summarizes some observations on the composition of flower-buds, and attention may be drawn to the high nitrogen-contents, equivalent to 20–28% crude protein in the dry matter of the complete buds, and reaching 38% in the ovules at flowering. The reproductive parts of the plant contain a much higher proportion of nitrogen than the vegetative organs (Maskell & Mason, 1930, etc.), and the diet of the young larvae appears in fact to represent the richest available source of protein.

TABLE 7. *Observations on the composition of cotton flower-buds*

	Mean fresh weight (g.)	Percentage fresh weight			
		Water	Crude protein	Reducing sugars	Oligo-saccharides
Buds—younger	0·27	79·0	5·8	0·9	0·3
Buds—older	0·69	80·5	4·9	0·7	0·5
Flowers—carpels	0·47	78·6	4·0	—	—
Flowers—ovules	0·03	78·3	7·9	0·9	0·7

Collected April–May 1939; variety 052, planted 7. xii. 1938.

The later instars also fed to an appreciable extent on rather older bolls; thus 20% of the 4th, 5th, and 6th instar larvae, as compared with 3% of the 1st and 2nd instars, were recorded as feeding on bolls 6–19 days old, at which stage the sugar-content is at a maximum. Parsons & Marshall showed furthermore that the larvae must have access to bolls of up to 3 weeks of age for the attainment of the full potential size of the pupa. Sugars are likely to be utilized in the formation of the fat which is accumulated during larval life, amounting on some diets to more than 40% of the dry weight at pupation (Ditman, 1938), and it has been shown for example in

tent caterpillars (*Malacosoma americana* F., Lasiocampidae) that the greater part of this fat is laid down during the later instars (Rudolfs, 1926). The larvae of the buff-tip moth (*Phalera bucephala* L., Notodontidae), feeding on hazel leaves, have been shown to utilize 80% of the soluble sugars as well as 60% of the protein in their food (Evans, 1939).

The feeding habits of the Sudan or red bollworm (*Diparopsis castanea* Hmps., Noctuidae) and the spiny bollworms (*Earias* spp., Noctuidae) resemble those of *Heliothis* on cotton. Thus *Diparopsis* feeds usually on buds, flowers or very young bolls during the first two instars, while the older larvae tend to select older fruit as food, though it is unusual to find larvae feeding in bolls in which the seed-coats are hardening and the lint beginning to dry out (Pearson & Mitchell, 1945). *Earias* shows a marked preference for flowers, buds and half-grown bolls, attacking both larger and smaller bolls to a lesser extent (Taylor & Gwynn, 1940); half-size bolls represent the stage of maximum sugar-content (Fig. 2).

The pink bollworm (*Platyedra gossypiella* Saund., Gelechiidae) also attacks buds, particularly when they are more numerous than bolls (Taylor, 1940), but differs from the other bollworms in feeding extensively in ripe seed, especially towards the end of the season. Squire (1937) observed diapause in this species in the West Indies only in larvae which had fed on seed which was almost or quite ripe, and never in larvae from flowers or young bolls. Larvae in diapause were reported to contain less moisture and more fat than short-cycle forms, and it was considered that diapause was induced by the low moisture-content and high oil-content of a diet of ripe seed (Squire, 1940*a*, *b*). Experiments on *Diparopsis* in Nyasaland on the other hand have shown that in this species age of food-supply has no appreciable effect on the induction of diapause (Pearson & Mitchell, 1945).

In conclusion, it must first be emphasized that the recorded analytical data relate only to some of the major boll constituents which are likely to be of nutritional interest. Further work on the nutrition of the insects involved will certainly necessitate more detailed studies of the dietary carbohydrates, fats and proteins. Accessory food factors, such as the sterols and members of the B-complex required by many insects (Fraenkel & Blewett, 1943, etc.), and inositol in the case of *Nematospora* (Buston & Pramanik, 1931), were likewise necessarily omitted from a preliminary investigation of this nature, but their potential importance must not be overlooked. Tannins also have been demonstrated in the healthy developing ovules (Rainey, 1940, 1941), and may similarly affect the incidence of pests and diseases.

Furthermore, no attempt is made in the present paper to deal with the role of morphological characters in the determination of susceptibility; the possibilities of this approach to problems of plant breeding are exemplified by the accuracy with which susceptibility to cotton jassid (*Empoasca facialis* Jac.) may be estimated from measurements of leaf hairs (MacDonald *et al.* 1945, etc.).

Despite such possible complications, the present observations show that con-

sideration of the changes in various constituents of the developing boll does in fact throw considerable light on the changes in susceptibility to pests and diseases occurring during the course of boll development.

I am greatly indebted to Mr F. R. Parnell and my Barberton colleagues for facilities and co-operation, and for the use of unpublished observations; to Dr E. J. Maskell, F.R.S., and Mr J. B. Hutchinson, C.M.G., for their advice and interest in the preparation of this paper; and to Mr Arnold Clark, of South African Pulp and Paper Industries Ltd., and Mr H. S. Hopf, of the Imperial College of Science and Technology, for information on the estimation of cellulose.

REFERENCES

BALLS, W. L. (1915). *The development and properties of raw cotton.* London: Black.

BOZA BARDUCCI, T., GARCIA RADA, G. & WILLE, J. E. (1945). Control of internal boll rot of the cotton plant caused by insect punctures (*Dysdercus* sp.), through selection of resistant strains. *Nature, Lond.*, **156**, 235.

BROWN, H. B. (1938). *Cotton* (2nd ed.). New York: McGraw-Hill.

BUSTON, H. W. & PRAMANIK, B. N. (1931). The accessory growth factor necessary for the growth of *Nematospora gossypii*. I. The chemical nature of the accessory factor. *Biochem. J.* **25**, 1656.

CASKEY, C. & GALLUP, W. D. (1931). Changes in the sugar, oil and gossypol content of the developing cotton boll. *J. agr. Res.* **42**, 671.

CHIBNALL, A. C. (1939). *Protein metabolism in the plant.* New Haven: Yale.

DASTUR, R. H. & AHAD, A. (1945). Studies in the periodic partial failures of the Punjab-American cottons in the Punjab. XV. Formation of proteins, oil and cellulose in the bolls of normal and tirak-affected plants. *Indian J. agric. Sci.* **15**, 75.

DITMAN, L. P. (1938). Metabolism in the corn ear worm. I. Studies on fat and water. *Bull. Md. agric. Exp. Sta.* **414**, 183.

DORÉE, C. (1933). *The methods of cellulose chemistry.* London: Chapman and Hall.

EVANS, A. C. (1938). Physiological relationships between insects and their host plants. I. The effect of the chemical composition of the plant on reproduction and production of winged forms in *Brevicoryne brassicae* L. *Ann. appl. Biol.* **25**, 558.

EVANS, A. C. (1939). The utilization of food by the larva of the Buff-tip, *Phalera bucephala* L. *Proc. R. ent. Soc. Lond.* A, **14**, 25.

FARRIES, E. H. M. & BELL, A. F. (1930). On the metabolism of *Nematospora gossypii* and related fungi, with special reference to the source of nitrogen. *Ann. Bot. Lond.* **44**, 423.

FRAENKEL, G. & BLEWETT, M. (1943). The natural foods and the food requirements of several species of stored products insects. *Trans. R. ent. Soc. Lond.* **93**, 457.

HOPKINS, J. C. F. (1932). Some diseases of cotton in Southern Rhodesia. *Emp. Cott. Gr. Rev.* **9**, 109.

HULME, A. C. & NARAIN, R. (1931). The ferricyanide method for the determination of reducing sugars. A modification of the Hagedorn-Jensen-Hanes technique. *Biochem. J.* **25**, 1051.

HUTCHINSON, J. B. (1940). The application of genetics to plant breeding. I. The genetic interpretation of plant breeding problems. *J. Genet.* **40**, 271.

HUTCHINSON, J. B. (1943). The effect of environment on hair characters and spinning value in Sea Island cotton. *J. Text. Inst.* **34**, T61.

JOSHI, K. V., GODE, R. B. & SHAH, A. K. (1941). Studies in the physiology of the Broach cotton plant. *Indian Central Cotton Committee*; *scientific monograph* no. 1.

MACDONALD, D., FIELDING, W. L. & RUSTON, D. F. (1940). Cotton breeding and field experiments. *Emp. Cott. Gr. Corp. Progr. Rep.* 1938–9, p. 15.

MacDonald, D., Fielding, W. L. & Ruston, D. F. (1941). Cotton breeding and field experiments. *Emp. Cott. Gr. Corp. Progr. Rep.* 1939–40, p. 12.

MacDonald, D., Fielding, W. L., Ruston, D. F. & King, H. E. (1946). Genetics and breeding. *Emp. Cott. Gr. Corp. Progr. Rep.* 1944–5, p. 15.

MacDonald, D., Ruston, D. F. & King, H. E. (1945). *Emp. Cott. Gr. Corp. Progr. Rep.* 1943–4, p. 25.

Martin, R. D., Ballard, W. W. & Simpson, D. M. (1923). Growth of fruiting parts in cotton plants. *J. agr. Res.* **25**, 195.

Maskell, E. J. & Mason, T. G. (1930). Studies on the transport of nitrogenous substances in the cotton plant. V. Movement to the boll. *Ann. Bot., Lond.*, **44**, 657.

Mason, T. G. & Maskell, E. J. (1928). Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark and wood, and of the effects of ringing. *Ann. Bot., Lond.*, **42**, 1.

Parsons, F. S. & Ullyett, G. C. (1936). Investigations on *Trichogramma lutea* Gir., as a parasite of the cotton bollworm *Heliothis obsoleta* Fabr. *Bull. ent. Res.* **27**, 219.

Parsons, F. S. & Marshall, J. (1941). Entomological work. *Emp. Cott. Gr. Corp. Progr. Rep.* 1939–40, p. 47.

Pearson, E. O. (1934). Preliminary observations on cotton stainers and internal boll disease of cotton in S. Africa. *Bull. ent. Res.* **25**, 383.

Pearson, E. O. (1937). Investigations on cotton stainers and internal boll disease. *Emp. Cott. Gr. Corp. Progr. Rep.* 1935–6, p. 37.

Pearson, E. O. (1939). Investigations on cotton stainers and internal boll disease. *Emp. Cott. Gr. Corp. Progr. Rep.* 1937–8, p. 42.

Pearson, E. O. (1947). The development of internal boll disease of cotton in relation to time of infection. *Ann. appl. Biol.* **34**, 527.

Pearson, E. O. & Mitchell, B. L. (1945). *A report on the status and control of insect pests of cotton in the Lower River districts of Nyasaland.* Zomba: Government Printer.

Rainey, R. C. (1940). Insect physiology. *Emp. Cott. Gr. Corp. Progr. Rep.* 1938–9, p. 40.

Rainey, R. C. (1941). Physiological work. *Emp. Cott. Gr. Corp. Progr. Rep.* 1939–40, p. 39.

Rudolfs, W. (1926). Some chemical information regarding apple tree tent caterpillar. *Rep. New Jersey agric. Exp. Sta.* 1924–25, p. 376.

Soyer, D. (1937). Les caractéristiques du cotonnier au Lomami. *Publ. Inst. Étude agron. Congo belge, Sér. tech.* no. 16.

Squire, F. A. (1937). A theory of diapause in *Platyedra gossypiella* Saund. *Trop. Agriculture*, **14**, 299.

Squire, F. A. (1940*a*). Observations on the larval diapause of the pink bollworm, *Platyedra gossypiella* Saund. *Bull. ent. Res.* **30**, 475.

Squire, F. A. (1940*b*). On the nature and origin of the diapause in *Platyedra gossypiella* Saund. *Bull. ent. Res.* **31**, 1.

Steyaert, R. L. (1938). Quelques données sur la physiologie de la capsule du cotonnier (en vue du problème de la stigmatomycose). *Inst. Étude agron. Congo belge, Sta. Bambesa.*

Steyaert, R. L. (1939). La sélection du cotonnier pour la résistance aux stigmatomycoses. *Publ. Inst. Étude agron. Congo belge, Sér. sci.* no. 16.

Taylor, T. H. C. (1940). Pink bollworm—*Platyedra gossypiella* Saund. *Agriculture in Uganda*, p. 260. Oxford.

Taylor, T. H. C. & Gwynn, A. M. (1940). Spiny bollworms—*Earias* spp. *Agriculture in Uganda*, p. 271. Oxford.

Walter, A. (1940). The climate of Uganda with special reference to the agricultural districts. *Agriculture in Uganda*, p. 14. Oxford.

Wickens, G. M. (1940). Plant pathology. *Emp. Cott. Gr. Corp. Progr. Rep.* 1938–9, p. 50.

Wickens, G. M. (1947). Plant pathology. *Emp. Cott. Gr. Corp. Progr. Rep.* 1945–6, p. 20.

(*Received* 18 *June* 1947)

PHYSICAL FACTORS AFFECTING THE TOXICITY OF SPRAYS TO STORED PRODUCT INSECTS

I. THE QUANTITY OF CARRIER IN WHICH A GIVEN AMOUNT OF ACTIVE INGREDIENT IS APPLIED

By P. S. HEWLETT

Pest Infestation Laboratory, Slough, Bucks

The problem of whether a given amount of active ingredient is more effectively applied in concentrated or dilute form is discussed. If y is percentage mortality and x_1 and x_2 are respectively log-concentration of active ingredient and log-volume (or deposit) of insecticide applied, y may be regarded as a uniform, continuous function of x_1 and x_2. The amount of active ingredient is constant, i.e. $x_1+x_2=k$, so that

$$\frac{dy}{dx_1}=-\frac{dy}{dx_2}=\frac{\partial y}{\partial x_1}-\frac{\partial y}{\partial x_2}. \qquad (4)$$

Probit mortality, Y, can be substituted for y in (4). Thus, whether an active ingredient is better applied in concentrated or dilute form depends on the relative magnitudes of $\partial y/\partial x_1$ and $\partial y/\partial x_2$, or of $\partial Y/\partial x_1$ and $\partial Y/\partial x_2$. Equation (4) is true whenever an insecticide consists of an active ingredient in a diluent, whatever the dosage-mortality relationship. Previous work is discussed in the light of (4) and its probit form, and it appears that the concentration at which an active ingredient is best applied can depend upon the nature and quantity of the active ingredient, and the method of application of the spray. There may be other factors.

The probit form of (4) is applied to the probit plane and confirmed experimentally. Flour beetles, *Tribolium castaneum* Herbst were sprayed with pyrethrins in Shell oil P 31, and it was found that $\partial Y/\partial x_1 > \partial Y/\partial x_2$, so that a given quantity of pyrethrins was more toxic in concentrated solution than in dilute.

Introduction

A spray used for the control of insects generally consists of an active ingredient, a substance or substances highly toxic to insects, dispersed in a carrier, which is of much lower toxicity. Most of the sprays now used in this country for the control of stored product insects consist of pyrethrins or synthetic organic compounds dissolved in a medium-heavy white mineral oil. The quantity of the carrier may affect the proportion of insects killed by the active ingredient. So also may the quality of the carrier, by its influence on the physical nature of the spray; for the active ingredient may be dispersed as a solution, emulsion, or suspension according to the carrier used, and, as the concentration of active ingredient is normally low, the carrier often largely determines such properties of the spray as viscosity and surface tension.

Environmental factors such as temperature, light, and atmospheric humidity may affect the toxicity of a spray. They may do this through their physiological effects on the insects, or through their effects on the physical properties of the spray, or through both. For example, an increase in temperature might influence the proportion of insects killed by increasing the metabolic rate of the insects, or by decreasing the

viscosity of the spray, or by both. The effects on toxicity of the physical properties of the spray and of the physical characteristics of the environment cannot properly be studied separately.

The problem considered in this paper is how the quantity of carrier affects the degree of control attained when a given amount of active ingredient is used; whether, in fact, a higher proportion of insects will be killed by applying a given quantity of active ingredient in concentrated or dilute form, in a small or large volume of spray. Would, for instance, 50 g. of D.D.T., under a given set of conditions, kill more insects if applied in 1 l. of a 5% solution or in 5 l. of a 1% solution?

The ranges of concentration of active ingredient and the volume of insecticide applicable during spraying operations against stored product insects are obviously restricted by considerations of cost, solubility, risk of staining or taint of stored goods, spraying equipment, and so on. Within limits, however, it is still possible to vary the concentration of active ingredient and amount of insecticide applied.

The relation between mortality, concentration, and volume of insecticide applied for a given amount of active ingredient used

In a given spraying operation, if other factors are constant, the percentage mortality in the population of insects will depend upon the concentration of active ingredient in the insecticide, and upon the volume of insecticide used, or some directly proportional variate, e.g. deposit under some circumstances. Hence percentage mortality (y) will be related to log-concentration (x_1) and log-volume or log-deposit (x_2). Furthermore, it is reasonable to suppose that only one value of y will correspond to each pair of values of x_1 and x_2, and that the population of insects will be large enough for the discontinuities in y due to the deaths of individual insects to be unimportant. Thus,

$$y = f(x_1, x_2), \tag{1}$$

where $f(x_1, x_2)$ is a uniform, continuous function in x_1 and x_2.

If the amount of active ingredient used is held constant, the concentration of active ingredient will vary inversely as the volume of insecticide prepared, i.e. the product of concentration and volume will be constant, so that

$$x_1 + x_2 = k. \tag{2}$$

By eliminating x_2 or x_1 from (1) by means of (2), y may be obtained as a function of x_1 or x_2 alone, giving rise to the differential coefficients dy/dx_1 and dy/dx_2. As x_1 and x_2 are related, from (1)

$$\frac{dy}{dx_1} = \frac{\partial y}{\partial x_1} + \frac{\partial y}{\partial x_2} \cdot \frac{dx_2}{dx_1}. \tag{3}$$

From (2), $dx_2/dx_1 = -1$, so that

$$\frac{dy}{dx_1} = -\frac{dy}{dx_2} = \frac{\partial y}{\partial x_1} - \frac{\partial y}{\partial x_2}. \tag{4}$$

The conditions governing changes in y when x_1 and x_2 are varied subject to (2) follow from (4). An increase in x_1 (and a consequent decrease in x_2) will increase or decrease y as dy/dx_1 is positive or negative, i.e. as $\partial y/\partial x_1 >$ or $< \partial y/\partial x_2$. Similarly, an increase in x_2 (and a consequent decrease in x_1) will increase or decrease y as $\partial y/\partial x_2 >$ or $< \partial y/\partial x_1$. Changes in x_1 and x_2 will leave the value of y unaltered so long as $\partial y/\partial x_1 = \partial y/\partial x_2$. Thus, for a given amount of active ingredient, changes in the concentration of active ingredient and the volume of spray used will affect the proportion of insects killed according to the relative magnitudes of $\partial y/\partial x_1$ and $\partial y/\partial x_2$. The values of dy/dx_1 and dy/dx_2 are not necessarily constant, and may change from positive to negative, or vice versa, as x_1 and x_2 increase and decrease subject to (2); i.e. the percentage mortality may reverse the direction of its change as x_1 is increased—it may for instance first increase and then decrease. In many cases, however, the mortality can be expected to change progressively in one direction over wide ranges of values as x_1 increases.

A similar line of reasoning can be followed if y is replaced by a variate completely and positively correlated, e.g. probit mortality. Probit mortality, Y, may therefore be substituted for y in (4), giving

$$\frac{dY}{dx_1} = -\frac{dY}{dx_2} = \frac{\partial Y}{\partial x_1} - \frac{\partial Y}{\partial x_2}. \tag{5}$$

Conclusions similar to those from (4) may be drawn from (5).

Equations (1) and (2), and therefore (4) and (5), are true whether the carrier is toxic or not. If the carrier is not toxic (1) is merely subject to the restriction that $f(-\infty, x_2) = 0$.

Application of the theoretical considerations to the probit plane

As the probit plane, which has been discussed by Finney (1943), is likely to be of value in the analysis of toxicological data involving two dosage factors, it will now be shown how the above theoretical considerations apply to the probit plane.

The relevant probit plane is

$$Y = a + b_1 x_1 + b_2 x_2, \tag{6}$$

where x_1 and x_2 have the same meaning as before, and a_1, b_1, and b_2 are parameters. Here $\partial Y/\partial x_1 = b_1$ and $\partial Y/\partial x_2 = b_2$, so that from (5)

$$\frac{dy}{dx_1} = -\frac{dy}{dx_2} = b_1 - b_2. \tag{7}$$

Alternatively, eliminating x_2 or x_1 from (6) by means of (2),

$$Y = a + b_2 k + (b_1 - b_2)\, x_1, \tag{8}$$

$$Y = a + b_1 k + (b_2 - b_1)\, x_2, \tag{9}$$

from which (7) may be derived by differentiation. It is obvious from (8) and (9) that if x_1 and x_2 are varied subject to (2), plotting Y against x_1 or x_2 will result in a straight line of slope $\pm(b_1 - b_2)$. An increase in x_1 will increase or decrease

Y as $b_1 >$ or $< b_2$, and similarly an increase in x_2 will increase or decrease y as $b_2 >$ or $< b_1$. If $b_1 = b_2$, changes in x_1 and x_2 will leave Y unaffected. As x_1 varies in one direction, so will Y change progressively in one direction only.

In order to give some idea of how much the mortality due to a given amount of active ingredient may be influenced by varying the concentration at which it is applied, the mortalities to which a 50% mortality can be raised by doubling or halving the concentration, whichever is appropriate, have been tabulated below against different values of $(b_1 \sim b_2)$. It has been assumed that a probit plane relation holds, so that the percentage mortalities correspond to $[5 + (b_1 \sim b_2) \log 2]$ probits.

$(b_1 \sim b_2)$	By doubling or halving concentration, 50% mortality raised to
1	62%
2	73%
4	89%
6	96%
10	99·9%

Experimental verification of the theoretical predictions

The theoretical expectations were shown to be fulfilled in a laboratory experiment in which flour beetles, *Tribolium castaneum* Herbst, were sprayed with pyrethrum in Shell oil P31 by the method described by Hewlett (1947). The mortalities thus obtained can be fitted to a probit plane (see Hewlett, 1947). In this experiment x_1 and x_2 were respectively log-(concentration expressed as % w/v total pyrethrins) and log-(deposit of spray in mg./10 sq.cm.). The experiment was devised so as (*a*) to determine the constants of the probit plane and (*b*) to vary concentration inversely as deposit for $k = 0{\cdot}879$. This value of k was chosen so as to obtain suitable mortalities with convenient concentrations and deposits. The results are given in Table 1: the sprayings which contributed to the plane (P) and those for which concentration was varied inversely as deposit (L) are indicated in the column headed 'Contribution of spraying'. The sprayings which contributed to the 'L' series only were not included in the calculation of the plane because of the difficulty of assigning expected probits to them.

The 6-day data marked 'P' were fitted to a probit plane by the method recommended by Finney (1943), and the values of b, with their standard errors, were found to be as follows:

$$b_1 = 7{\cdot}88 \pm 0{\cdot}98,$$

$$b_2 = 3{\cdot}66 \pm 0{\cdot}51,$$

$$b_2 - b_1 = -4{\cdot}22 \pm 0{\cdot}89.$$

The 6-day data marked 'L' were fitted to a line, $Y = a' + b'x_2$, as described by Bliss (1935, 1938), and it was found that

$$b' = -4{\cdot}01 \pm 0{\cdot}51.$$

Obviously $b' = b_2 - b_1$ within the limits of error, confirming the prediction embodied

TABLE 1. *The toxicity to* T. castaneum *of different concentrations and deposits of pyrethrins in Shell oil P31 applied as a direct spray: each figure for mortality was obtained from a batch of 49–50 insects*

Pyrethrin concentration (% w/v)	Estimated deposit (mg./10 sq.cm.)	Mortality % after 6 days	Mortality % after 9 days	Contribution of spraying*
0·40	18·9	4	8	*L*
0·60	12·6	6	14	*L*
1·00	3·50	0	4	*P*
	3·50	10	16	*P*
	7·55	36	50	*L*
1·20	3·10	8	16	*P*
	3·50	10	22	*P*
	3·50	18	20	*P*
	3·90	18	30	*P*
	4·85	48	54	*P*
	6·31	44	54	*P, L*
	6·31	52	66	*P, L*
	7·96	55·1	73·5	*P*
	10·0	72	80	*P*
	12·4	88	92	*P*
	15·9	90	92	*P*
1·60	3·50	24·5	34·0	*P*
	4·73	61·2	73·2	*L*
2·16	3·50	91·8	93·9	*P, L*
	3·50	94	100	*P, L*
2·70	2·80	86	98	*L*
	3·50	94	94	*P*
	3·50	94	100	*P*
3·60	2·10	94	98	*L*
Control	—	0·5†	1·0†	—

* See text (p. 87). † Figures from 200 insects.

in equations (7) and (9), since $b' = dY/dx_2$. Although, owing to heterogeneity, their errors were larger than had been hoped, both $(b_2 - b_1)$ and b' clearly differed significantly from zero. Hence a given quantity of pyrethrins was more toxic to *T. castaneum* as a direct spray when in concentrated solution in P31 than when in dilute. The 9-day data were not analysed statistically, but a graphical analysis indicated that

$$b_1 \approx 7{\cdot}95,$$
$$b_2 \approx 3{\cdot}62,$$
$$b_2 - b_1 \approx -4{\cdot}33,$$
$$b' \approx -4{\cdot}43.$$

The errors were evidently of the same order as those for 6 days, so that the theoretical predictions received additional confirmation.

It is evident from Table 1 that throughout the series of mortalities marked '*L*' there was increase in mortality as the concentration of pyrethrins increased.

Discussion

Although equations (4) and (5) have been derived in considering the control of stored product insects by sprays, these equations can be more widely applied in the study of insecticides. The equations can be applied to the effects of any insecticidal preparation consisting of an active ingredient and a diluent, where $x_1 =$ log-concentration of active ingredient and $x_2 =$ log-amount of insecticide used. They may, for instance, be used for the analysis of the results of toxicity tests with the usual types of flysprays, and with 'chemical' (as opposed to 'inert mineral') dusts, whether the active ingredient of the dust is sorbed on the carrier or not. By putting $x_1 =$ log-concentration and $x_2 =$ log-time, the equations may be used to decide whether a fumigant is more effective at a high concentration for a short exposure, or at a low concentration for a long exposure. Equations (4) and (5) are true whatever the dosage-mortality relationship, so long as it may be considered uniform and continuous.

Under most circumstances, an increase in the concentration of active ingredient for a given amount of insecticide used, or an increase in the amount of insecticide for a given concentration, will cause an increase, or at least no decrease, in mortality; i.e. $\partial y/\partial x_1$ and $\partial y/\partial x_2 \geqslant 0$. If $\partial y/\partial x_1$ and $\partial y/\partial x_2 \geqslant 0$, the absolute values of dy/dx_1 and dy/dx_2 can never be larger than $\partial y/\partial x_1$ or $\partial y/\partial x_2$, whichever is the greater. Exceptionally $\partial y/\partial x_2$ may be negative. When, for instance, in horticultural spraying the volume of spray applied is increased beyond the 'drip-point', less spray adheres to the leaves of the sprayed plants than if the drip-point had not been passed, and the proportion of insects killed may be lowered.

The problem of whether a given amount of active ingredient is better applied in concentrated or dilute form seems to have attracted more attention in relation to dusts than in relation to sprays. Turner (1945), working on dusts, found that a given amount of active ingredient ('toxicant') was more effective when applied at high concentration, but quoted some work which agreed with his own, and other work which did not. Turner attempted to explain these results by differences in 'coverage', but made the unjustifiable assumption that a given amount of active principle would have the same physiological effect on an insect irrespective of the dilution at which it reached the insect. Logically, however, the first step in the problem is the resolution of dy/dx_1 and dy/dx_2 into their components $\partial y/\partial x_1$ and $\partial y/\partial x_2$, the values of which may be affected by coverage. Turner (1945) gave numerical data for the toxicity of derris-pyrophyllite dust to the Mexican bean beetle, *Epilachna varivestis* Muls., and showed his data graphically in his figs. 2 and 3. In both figures he plotted Y against log (lb. rotenone/acre), i.e. $(x_1 + x_2 - 2)$, so that the slopes of the lines in fig. 3 are $\partial Y/\partial x_1$ and those of the lines in fig. 2 are $\partial Y/\partial x_2$. It is evident from the figures that, for any given mortality in the range observed, $\partial Y/\partial x_1 > \partial Y/\partial x_2$, so that from equation (5) a given amount of active principle would be more effective at a high concentration. This is obvious when Turner's numerical data are read diagonally.

Lindquist *et al.* (1945) tested D.D.T.-in-kerosene sprays against house-flies, *Musca domestica* L., and mosquitoes, *Anopheles quadrimaculatus* Say. They varied the concentration of D.D.T. between 1 and 16%, and varied the volume of spray inversely as the D.D.T. concentration. The concentrated and dilute solutions were equally effective. On the same basis they found that a spray containing 10% D.D.T. + 1% pyrethrins was as effective as one containing 20% D.D.T. + 2% pyrethrins. It seems impossible to tell with certainty from published data whether pyrethrins are more toxic to *Musca domestica* when applied in concentrated or in dilute solution. Several workers have related mortality to concentration of pyrethrins, but very few have related mortality to the volume of spray atomized. Ford (1941) gives toxicity data from Peet-Grady tests for 0·10 and 0·15% pyrethrins in deodorized kerosene, at doses of 6 and 12 ml., and exposure times of 5 and 10 min. His figures have been analysed on the assumption that probit mortality was linearly related to log-concentration of pyrethrins and log-volume of insecticide. Other workers have found that probit mortality of house-flies was linearly related to log-concentration of pyrethrins (see Bliss, 1939). The values of b_1 and b_2 calculated from Ford's data were as follows:

		5 min. exposure	10 min. exposure
b_1	6 ml.	2·15	1·41
	12 ml.	1·57	2·22
b_2	0·10% pyrethrins	1·21	0·88
	0·15% pyrethrins	0·91	1·35

As the values for b_1 were a little higher than those for b_2, pyrethrins may be slightly more toxic to *M. domestica* in concentrated solution than in dilute.

Callaway & Musgrave (1940) sprayed eggs of the bed-bug, *Cimex lectularius* L., with β-butoxy-β'-thiocyanodiethyl ether and with pyrethrins in odourless distillate. Examination of their data shows that a given quantity of the former active ingredient was more toxic in 3% solution than in 2%, but a given quantity of the latter was more toxic in 0·1% solution than in 0·4%.

Robinson (1942) exposed ticks, *Ornithodorus moubata* Murray, on films formed by pyrethrins in Shell oil 24210 on Whatman no. 2 filter papers. He found that smaller quantities of pyrethrins gave higher mortalities if applied at low concentrations, but larger quantities gave higher mortalities at high concentrations.

For sprays in which P31 is the carrier, there is a considerable amount of data to show whether a given amount of active ingredient is better applied in concentrated or dilute solution. Robinson (1943) exposed *O. moubata* on pyrethrum films formed on plaster. He found that a given amount of pyrethrins was more toxic in a spray containing about 0·82% pyrethrins (0·41% pyrethrin I) than in one containing half that concentration of pyrethrins. Tattersfield & Potter (1943) exposed flour beetles, *Tribolium castaneum*, on pyrethrum films on Whatman no. 544 filter papers and obtained results which could be fitted to a probit plane, with $b_1 > b_2$. When

TABLE 2. *The effect on mortality of increasing the concentration of active ingredient in sprays when a given amount of active ingredient is used*

Case no.	Test species	Source of information	Active ingredient	Carrier	Method of application	Mortality
1	*M. domestica*	Lindquist *et al.* (1945)	D.D.T.	Kerosene	Mist	Unaffected
2	*M. domestica*	Lindquist *et al.* (1945)	D.D.T. + pyrethrins	Kerosene	Mist	Unaffected
3	*M. domestica*	Ford (1941)	Pyrethrins	Deodorized kerosene	Mist	Increased
4	*A. quadrimaculatus*	Lindquist *et al.* (1945)	D.D.T.	Kerosene	Mist	Unaffected
5	*A. quadrimaculatus*	Lindquist *et al.* (1945)	D.D.T. + pyrethrins	Kerosene	Mist	Unaffected
6	Eggs of *C. lectularius*	Callaway & Musgrave (1940)	β-Butoxy-β′-thio-cyano-diethyl ether	Odourless distillate	Direct spray	Increased
7	Eggs of *C. lectularius*	Callaway & Musgrave (1940)	Pyrethrins	Odourless distillate	Direct spray	Decreased
8	*O. moubata*	Robinson (1942)	Pyrethrins	Shell oil 24210	Film on Whatman no. 2 filter paper	Decreased* or increased†
9	*O. moubata*	Robinson (1943)	Pyrethrins	Shell oil P31	Film on plaster	Increased
10	*T. castaneum*	Tattersfield & Potter (1943)	Pyrethrins	Shell oil P31	Film on Whatman no. 544 filter paper	Increased
11	*T. castaneum*	Hewlett (1947)	Pyrethrins	Shell oil P31	Film on Whatman no. 544 filter paper	Decreased
12	*T. castaneum*	Hewlett (unpublished)	D.D.T.	Shell oil P31	Film on Whatman no. 544 filter paper	Decreased
13	*T. castaneum*	Hewlett (unpublished)	Gammexane	Shell oil P31	Film on Whatman no. 544 filter paper	Increased
14	*T. castaneum*	Tattersfield & Potter (1943)	Pyrethrins	Shell oil P31	Direct spray	Increased
15	*T. castaneum*	Hewlett (1947 and present paper)	Pyrethrins	Shell oil P31	Direct spray	Increased

* For smaller quantities of pyrethrins. † For larger quantities of pyrethrins.

T. castaneum were exposed on films on the same type of filter paper by the technique of Parkin & Green (1943), for pyrethrins $b_2 > b_1$ (Hewlett, 1947); for D.D.T. $b_2 > b_1$ (Hewlett, unpublished); and for Gammexane $b_1 > b_2$ (Hewlett, unpublished). When *T. castaneum* were directly sprayed with pyrethrins in P31, both Tattersfield & Potter (1943) and Hewlett (1947, and present paper) found that $b_1 > b_2$. If $b_1 > b_2$, the active ingredient is more toxic in concentrated solution, and if $b_2 > b_1$, it is more toxic in dilute.

The conclusions from the part of the preceding discussion relating to sprays are summarized in Table 2. They are not, of course, as general as the right-hand column of the table implies, but are restricted as indicated in the discussion.

Some of the factors that may determine whether a given amount of active ingredient is more effectively applied in concentrated or dilute form are discernible from Table 2. Comparison of case 6 with 7, and of 11 with 12 and with 13, shows that the nature of the active ingredient is one factor. Case 8 shows that the quantity of active ingredient applied is a second. Comparison of case 11 with 10, 14, and 15 shows that the method of application of the spray is a third.

There may be other factors not indicated by Table 2. The species of insect is probably one of these, for the actual, and therefore the relative, magnitudes of $\partial y/\partial x_1$ and $\partial y/\partial x_2$ are likely to differ from species to species. The type of carrier is another possible factor.

The problem considered in this paper has been approached theoretically. Whether, under a given set of conditions, an active ingredient is better applied in concentrated or dilute form has been shown to depend upon the relative magnitudes of $\partial y/\partial x_1$ and $\partial y/\partial x_2$. The advantage of this theoretical approach over an empirical one is twofold. First, it shows that an active ingredient may be better applied at one concentration than at another because within an insect population the distribution of resistance with respect to concentration of active ingredient can differ from that with respect to the amount of insecticide applied. Secondly, whether an active ingredient is better applied in concentrated or dilute form can be decided from data not specially designed for this purpose. By making use of the latter fact, a larger body of data than would otherwise have been possible has been analysed to reveal some factors that can affect the concentration at which an active ingredient is best applied. In continuing this investigation a watch will therefore be kept for other physical factors of this kind.

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research. Miss B. Clayton assisted in the experimental work and computation.

REFERENCES

Bliss, C. I. (1935). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

Bliss, C. I. (1938). The determination of the dosage-mortality curve from small numbers. *Quart. J. Pharm.* **11**, 192.

Bliss, C. I. (1939). Fly spray testing: a discussion on the theory of evaluating liquid household insecticides by the Peet-Grady method. *Soap sanit. Chem.* **15** (4), 103.

Callaway, S. & Musgrave, A. J. (1940). Laboratory tests with liquid insecticides on the eggs of the bed-bug, *Cimex lectularius* L. *Ann. appl. Biol.* **27**, 252.

Finney, D. J. (1943). The statistical treatment of toxicological data relating to more than one dosage-factor. *Ann. appl. Biol.* **30**, 71.

Ford, J. H. (1941). Variation in Peet-Grady tests. Study of effects of using reduced dosage and reduced time exposure in the Peet-Grady test. *Soap sanit. Chem.* **17** (11), 91.

Hewlett, P. S. (1947). A direct spray technique for the biological evaluation of pyrethrum-in-oil insecticides for use against stored product insects in warehouses. *Ann. appl. Biol.* **34**, 357.

Lindquist, A. W., Schroeder, H. O. & Knipling, E. F. (1945). Concentrated insecticides. Preliminary studies of the use of concentrated sprays against house-flies and mosquitoes. *Soap sanit. Chem.* **21** (7), 109.

Parkin, E. A. & Green, A. A. (1943). A film technique for the biological evaluation of pyrethrum-in-oil insecticides for use against stored product insects in warehouses. *Ann. appl. Biol.* **30**, 279.

Robinson, G. G. (1942). The quantitative interaction of spray fluid and active principle in determining the toxicity of a pyrethrum preparation to the argasid tick, *Ornithodorus moubata* Murray. *Ann. appl. Biol.* **29**, 290.

Robinson, G. G. (1943). The use of a plaster substratum for testing pyrethrum-oil films against *Ornithodorus moubata* Murray (Acarina: Argasidae). *Bull. ent. Res.* **34**, 269.

Tattersfield, F. & Potter, C. (1943). Biological methods of determining the insecticidal values of pyrethrum preparations (particularly extracts in a heavy oil). *Ann. appl. Biol.* **30**, 259.

Turner, N. (1945). The coverage factor in the application of dusts. *J. econ. Ent.* **38**, 359.

(*Received* 10 *July* 1947)

THE EFFECTS OF D.D.T. AND OF BENZENE HEXACHLORIDE ON BEES

By M. J. WAY and A. D. SYNGE
Rothamsted Experimental Station, Harpenden, Herts

(With 3 Text-figures)

Laboratory and field experiments were carried out during 1946 to determine the effects of D.D.T. and of benzene hexachloride (B.H.C.)* on honey-bees and on several wild bee species.

Laboratory experiments show that B.H.C. is a powerful contact and stomach poison and results of field experiments with commercial preparations confirm its danger to the foraging bee population. A few minutes' contact with treated surfaces is sufficient to cause death and blossom may remain poisonous for at least 3 days after treatment.

In the laboratory D.D.T. has a contact action at fairly high concentrations and as a stomach poison is rather more toxic than lead arsenate. In the field, commercial preparations on open blossom are apparently harmless to foraging bees.

Introduction

As both D.D.T. and B.H.C. have unique properties of persistence and action as both contact and stomach poisons their widespread application suggests a greater danger to bees and other pollinating insects than that expected from the use of other insecticides. All previous work reported on this problem has been with the honey-bee and the first experiments carried out with D.D.T. by Weissmann (1942), Holst (1944) and Filmar & Smith (1944) showed that in the laboratory D.D.T. acts as both a contact and stomach poison to honey-bee workers. As a result of preliminary trials Weissmann suggested that Guesarol E spray was more powerful as a contact insecticide than as a stomach poison, but detailed repetition of the work by Holst produced contradictory results showing that D.D.T. in Guesarol E was a contact insecticide at 1 % but not at 0·05 %, while fed in syrup it acted as a stomach poison at 0·05 %. Quantitative data on the contact effect of D.D.T. films were obtained by Filmar & Smith who found that 20–30 min. exposure to deposits on glass plates varying from 0·01 to 0·4 mg. D.D.T./sq.in. gave a high kill within 34 hr.

A series of experiments on the effect of D.D.T. on honey-bees was carried out by Eckert (1945). Treatment of the combs of a hive with 3 % D.D.T. dust showed no permanent injury, whereas similar treatment with 20 % D.D.T. caused considerable irritation resulting in destruction of the queen and removal of the larvae, but apparently not causing any undue loss of workers or nurse bees. No injurious effects were observed on workers or larvae when 110 g. of pollen paste containing 0·25 g.

* The letters B.H.C. throughout the paper refer to crude benzene hexachloride or more correctly hexachlorcyclohexane. This consists of a mixture of isomers of which only the γ-isomer has a significant insecticidal action. Where the γ-isomer is specifically referred to γ is used as a prefix, thus γ-B.H.C.

D.D.T. were fed to a hive. Experiments on stomach-poison action in which workers were fed individually with 'colloidal' D.D.T. in syrup showed the L.D. 50 to be about 0·0046 mg./bee.

The only previous work reported on B.H.C. is by Cherian & Mahadevan (1946) with the Indian bee, *Apis indica*. Dusting of the inside of a hive with 1%* Gammexane dust caused complete mortality in 6 days while with 6%* dust all bees were dead in 10 hr. D.D.T. dusts containing less than 5% D.D.T. were not injurious but 10% D.D.T. had the same effect as 5%* Gammexane dust. Preliminary feeding trials were carried out in which the insecticides mixed with honey were poured over the combs of a hive. A $\frac{1}{4}$ oz. of honey containing 0·05% D.D.T. fed daily to a hive caused 96·5% kill after 7 days. In another hive treated similarly with 0·05%* Gammexane the kill reached 24% in 6 days after which the remaining bees gradually disappeared.

The present work was carried out primarily with the honey-bee, *Apis mellifera*, but, where possible, observations were also made on various wild bee species. Laboratory experiments were carried out which provided quantitative data on relative toxicities and also gave some indication of the extent of toxicity of D.D.T. and B.H.C. under rather severe conditions. Since laboratory conditions are artificial and results often misleading when applied to field conditions, outdoor experiments were also carried out in which observations were made on bees visiting treated blossom under natural conditions. These produced results which are considered to be of the greatest importance in assessing the toxicities of D.D.T. and B.H.C. in the field. Unfortunately, owing to bad weather conditions, field experiments were not so extensive as was hoped.

Materials and methods in laboratory experiments

Insecticides

The spray materials used were commercial dispersible powders. The concentrations of insecticide spray are shown as percentage weight/volume of toxic material, i.e. g. toxic material per 100 c.c. water, the percentage of carrier material being disregarded. Where dusts were used the concentrations are shown as percentage weight/weight of toxic material, i.e. g. toxic material per 100 g. dust.

Commercial dispersible powders, because of their particle size and high proportion of carrier material, were unsatisfactory for feeding experiments and when these were carried out laboratory preparations were used, details of which are given later.

Source of insect material

The *Apis mellifera* workers were all collected from the same colony and, unless otherwise stated, were taken from the feed-hole of the hive to obtain a representative

* It is not clear whether the percentages of Gammexane refer to % γ-B.H.C. or % total B.H.C.; probably the latter because concentrations of 1 and 5% γ-isomer in dusts would be abnormally high.

sample of the adult worker bee population. Wild bees were collected from whatever source was available at the time, details being given with each experiment.

Treatment of test insects

When anaesthetization was necessary bees were enclosed for a few minutes in a bell jar containing carbon dioxide. Recovery from anaesthesia was rapid and no ill effects were observed.

Two sizes of cages were used, the smaller 8 × 8 × 4 cm. holding ten bees and the larger 11 × 11 × 5 cm. holding fifteen bees. Fig. 1 shows a cross-section of a cage. Four walls were of wood with gauze-covered ventilation holes (*v*) and an aperture (*ap*) fitted with a cork for addition or removal of bees. The two large walls were of glass. The feeder (*f*) providing a continuous supply of syrup consisted of a test-tube containing 40% sucrose inverted over a watch-glass fixed to the floor of the cage. During all experiments, caged bees were kept in a dark incubator at 32° F. and about 45% relative humidity.

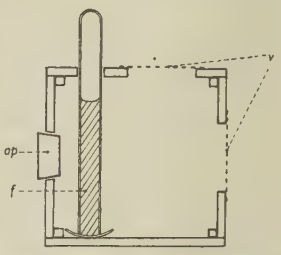


Fig. 1. Section of cage used in laboratory experiments with bees.

Behaviour of test insects

Apis mellifera workers can live under these conditions for over a fortnight though it was never found necessary to continue experiments for more than about a week. Deaths in the controls were generally very low in the first few days, but rose to an average of 20% on the eighth day. Much of this was probably due to age since in an experiment where young bees were selected no deaths were recorded in the controls until the ninth day.

Although the conditions would appear satisfactory, in one respect *A. mellifera* workers did not behave normally. Defecation was inhibited and in some individuals faeces collected in the rectum and caused distension of the abdomen, though otherwise the bees appeared normal and healthy. However, this is apparently the natural behaviour of bees confined to the hive. Wild bees defecated normally and several species kept in the laboratory for at least a fortnight remained in good condition.

Experiments were carried out over a period of 8 months during 1946 and data were obtained on variation in resistance of *A. mellifera* workers throughout this period. One experiment carried out in the spring on old overwintering bees and repeated in late summer on relatively young bees showed little material difference in resistance to insecticides and throughout a remarkable uniformity was noticeable.

Laboratory contact-poison experiments with adult workers of *Apis mellifera* and several bumble-bee species

Methods

The toxicity of several commercial preparations of D.D.T. and B.H.C. as contact poisons was determined in two ways: (1) by direct spraying and dusting of bees, (2) by a film technique in which worker bees were kept in contact with treated glass surfaces.

An Aerograph M.P. paint sprayer and a small hand-duster were used for applying the insecticides.

Technique and experimental procedure

In direct spraying and dusting experiments with *A. mellifera* workers two replicates of fifteen bees were used for each treatment. Batches of fifteen anaesthetized bees were placed in 9 cm. Petri dishes and heavily sprayed or dusted with the required concentrations of insecticide. The bees were then caged and immediately placed in the incubator. Bees sprayed with distilled water were used as controls.

Three experiments were carried out with bumble bees. For the first, bees were collected from the field and consisted of several species, the majority of which were drones of *Psithyrus vestalis* and *Bombus lucorum*. For two further experiments, queens, drones and workers were collected from nests of *B. agrorum* and *B. terrestris*. The technique was the same as that described for *Apis mellifera* except that the numbers of insects used per treatment were variable.

To test the effect of insecticide films the 8×8 cm. glass walls of a small cage were removed, sprayed with the required insecticide, the films allowed to dry and the glass walls replaced. Ten bees were placed in each cage, and for each treatment three cages were used in which the bees were kept until the end of the experiment.

Analysis of results

Table 1 shows the first day percentage kills of various bee species caused by direct spraying and dusting with commercial preparations of D.D.T. and B.H.C. Second and third day kills were not significantly higher than those obtained on the first day.

Results show that the B.H.C. preparations were considerably more toxic than D.D.T. to all species. Small numbers of insects were used in some of the bumble-bee experiments and the percentage kills were only determined in order to facilitate comparison, but the data are sufficient to show that workers of *Bombus agrorum* and *B. terrestris* have a susceptibility comparable to that of *Apis mellifera* workers, while the queens and drones are more resistant.

The data obtained from probit analysis of the results of direct spraying experiments with *A. mellifera* are shown in Table 2. The calculated concentrations of toxic material in g./100 c.c. required to give 20, 50 and 95% kills are included.

TABLE 1. *First day percentage kills of various bee species caused by direct spraying and dusting with commercial preparations of D.D.T. and B.H.C.*

Treatment	Concentrations of toxic material (% D.D.T.)	*A. mellifera* ♀	Mixed bumble-bee species mainly ♂	*B. agrorum* ♀	*B. terrestris* ♀	*B. terrestris* ♂	*B. terrestris* ♀
Guesarol E spray	1	—	30	—	20	33	100
	0·5	97	30	—	0	0	100
	0·25	93	—	—	—	—	—
	0·2	63	11	—	0	0	55
	0·15	43	—	—	—	—	—
	0·1	33	6	—	—	—	17
Guesarol dust	5	67	20	—	—	—	—
Controls	—	10	10	—	0	0	0
	(% γ-isomer)						
B.H.C. P530 spray	0·013	—	—	100	—	—	100
	0·0065	100	—	—	43	100	—
	0·0032	87	—	80	—	—	93
	0·0026	96	—	—	—	—	—
	0·0020	86	—	—	—	—	—
	0·0016	59	—	—	—	—	—
	0·0013	34	—	—	—	—	—
	0·0010	40	—	—	—	—	—
	0·0008	7	—	0	0	0	47
	0·00064	3	—	—	—	—	—
	0·00057	7	—	—	—	—	—
B.H.C. P.P. flea beetle dust	0·2	100	—	—	—	—	—
Controls	—	0	—	0	0	0	13
Number of bees per treatment		30	18–20	10	5–8	5–10	10–20

TABLE 2. *Probit analysis showing calculated concentrations of D.D.T. and γ-B.H.C in commercial sprays required to give* 20, 50 *and* 95% *kill of* Apis mellifera *workers by contact poisoning*

Insecticide	Regression equation	χ^2	Standard error of *b*	Lethal conc.-g. per 100 c.c. 20%	50%	95%
D.D.T. Guesarol E spray	$y=8{\cdot}39+4{\cdot}28x$	5·67	$4{\cdot}28\pm0{\cdot}92$	0·10	0·16	0·39
B.H.C. P530 spray	$y=17{\cdot}68+4{\cdot}43x$	23·0	$4{\cdot}43\pm0{\cdot}47$	0·00089	0·0014	0·0032

Experiments using the film technique were carried out with *A. mellifera* and the percentage kills on the first and fifth days are shown in Table 3.

First day kills suggest that under these conditions B.H.C. is approximately 100 times more toxic than D.D.T. The data are insufficient to determine the significance of the increase in kill after the first day. This experiment was not strictly quantitative because the weights of toxic material deposited on the cage walls were not necessarily equivalent to their concentrations in the sprays used.

TABLE 3. *Percentage kills of* Apis mellifera *workers on the first and fifth days caused by D.D.T. and B.H.C. films on glass*

	Treatment (% D.D.T.)	Percentage kill	
		First day	Fifth day
Guesarol E spray	1	100	—
	0·5	93	100
	0·2	37	67
	0·1	13	37
Control	—	5	15
	(% γ-isomer)		
B.H.C. P530 spray	0·0065	100	—
	0·0032	100	—
	0·0016	30	33
	0·0004	3	10
Control	—	0	3

LABORATORY STOMACH-POISON EXPERIMENTS WITH ADULT WORKERS OF *APIS MELLIFERA*

Materials

Pure samples of the toxic material were used to prepare suspensions in water of D.D.T., γ-B.H.C. and lead arsenate. Suspensions of crystalline D.D.T. and γ-B.H.C. were prepared using a technique described by McIntosh (1947). The medium contained 5% v/v acetone, 0·1% w/v sulphonated lorol powder and 40% w/v sucrose with the D.D.T. and γ-B.H.C. in the form of crystals of a uniform shape and size, those of the former being flat plates $60 \times 15\,\mu$. and the latter bifurcated needles $60\,\mu$. in length.

A suspension of 'colloidal' D.D.T. consisting of particles about $\frac{1}{2}\,\mu$. in diameter was prepared in a medium containing 10% v/v ethyl alcohol, 0·05% v/v sulphonated lorol liquid T.A., and 40% w/v sucrose.

The lead arsenate consisted of particles, about $10\,\mu$. in diameter, suspended in a medium containing 1% w/v casein as a suspensory agent (Butler, Finney & Schiele, 1943) and 40% w/v sucrose.

The required concentrations were obtained by dilution with the appropriate medium.

Technique and experimental procedure

Fig. 2 shows the apparatus used to feed individual worker bees with a known volume of insecticide. It consists of a length of capillary tubing (*c*) of known bore lying over a centimetre scale (*s*), the volume in the capillary over a distance of 3 cm. being 0·0122 c.c. The capillary filled to about three-quarters of its length with the required insecticide is attached to the scale by means of two rubber bands (*rb*) and

the insecticide column adjusted so that one meniscus (m_1) is flush with the projecting tip of the capillary while the other (m_2) lies over a scale division.

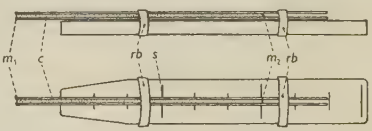


Fig. 2. Side and surface views of apparatus for feeding individual bees with known doses of insecticide.

Bees are starved for $\frac{1}{2}$ hr., anaesthetized, and to facilitate handling, are attached to glass rods (Frings, 1944). The procedure is to dip the end of a rod in warm beeswax and immediately press it against the dorsal surface of the thorax of the bee. The wax sets in a few seconds fixing the bee firmly to the rod.

After allowing a few minutes for the bee to recover from anaesthesia the proboscis is extended by means of a mounted needle and inserted in the projecting end of the feeding capillary. Sucrose in the medium immediately stimulates feeding. When the insecticide has been withdrawn over a length of 3 cm. (i.e. 0·0122 c.c. consumed) the bee is removed, detached from the glass rod and caged. The capillary is then refilled and the procedure repeated with another bee.

By means of this technique any contact action of the insecticide is limited to the tip of the proboscis and it is considered that the effects observed are those caused by the stomach-poison action.

Five or six concentrations of each insecticide were used and fifteen or thirty bees fed with each concentration.

Analysis of results

Table 4 shows the percentage kills of adult worker bees recorded on the first, third and fifth days after consumption of the insecticide. Dosages are shown as weight of active material per bee consumed.

TABLE 4. *Percentage kills of adult worker bees fed with suspensions of lead arsenate, D.D.T. and γ-B.H.C.*

		Active material consumed per bee mg. × 10^{-3}														
Insecticide	Day	488·0	244·0	122·0	61·0	48·8	24·4	12·2	6·10	3·05	0·732	0·488	0·244	0·122	0·061	Controls
Lead arsenate	First	47	13	0	0	—	—	0	—	—	—	—	—	—	—	0
	Third	100	87	47	27	—	—	13	—	—	—	—	—	—	—	0
	Fifth	—	100	87	73	—	—	40	—	—	—	—	—	—	—	0
D.D.T. crystals 60 × 20 μ.	First	—	—	—	—	100	100	60	27	7	—	—	—	—	—	7 0 } Two replicates
	Third	—	—	—	—	—	—	67	27	7	—	—	—	—	—	7 0 }
	Fifth	—	—	—	—	—	—	73	37	7	—	—	—	—	—	27 0 }
'Colloidal' D.D.T.	First	—	—	—	—	100	100	100	100	47	—	—	—	—	—	0
	Third	—	—	—	—	—	—	—	—	47	—	—	—	—	—	0
	Fifth	—	—	—	—	—	—	—	—	47	—	—	—	—	—	0
γ-B.H.C. crystals	First	—	—	—	—	—	—	—	—	—	100	100	73	63	43	7 0 } Two replicates
	Third	—	—	—	—	—	—	—	—	—	—	—	80	63	43	7 0 }
	Fifth	—	—	—	—	—	—	—	—	—	—	—	80	67	47	27 0 }

An examination of the table shows marked differences in toxicity of the three insecticides. D.D.T. and γ-B.H.C. are quick acting with no significant increase in

kill after the first day. 'Colloidal' D.D.T. appears to be more toxic than crystalline, while γ-B.H.C. by comparison is very highly toxic. First day kills by lead arsenate would suggest a relatively low toxicity but the speed of action is slow and even by the fifth day when toxicity was approaching that of D.D.T., maximum kill had not been attained.

Some of the data were subjected to probit analysis and the results are shown graphically in Fig. 3. The dosages of active material per bee required to give 20, 50 and 95% kills were determined and are shown in Table 5.

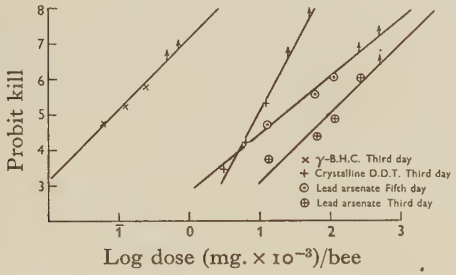


Fig. 3. Graph showing relative toxicities of lead arsenate, D.D.T. and γ-B.H.C. as stomach poisons to workers of *Apis mellifera*.

TABLE 5. *Probit analysis of results obtained by feeding* Apis mellifera *workers with suspensions of lead arsenate, D.D.T. and γ-B.H.C., including the calculated dosages required to give* 20, 50 *and* 95% *kill*

Treatment	Regression equation	Standard error of b	χ^2	Lethal dose mg. × 10⁻³ 20%	50%	95%
Lead arsenate, third day	$y=1{\cdot}26+1{\cdot}93x$	$1{\cdot}93\pm0{\cdot}40$	5·9	32·0	86·0	610·0
Lead arsenate, fifth day	$y=2{\cdot}88+1{\cdot}63x$	$1{\cdot}63\pm0{\cdot}35$	0·79	6·1	20·0	210·0
Crystalline D.D.T., third day	$y=1{\cdot}44+3{\cdot}72x$	$3{\cdot}72\pm0{\cdot}59$	1·09	5·4	9·1	25·0
γ-B.H.C., third day	$y=7{\cdot}17+1{\cdot}98x$	$1{\cdot}98\pm0{\cdot}39$	0·80	0·03	0·08	0·54

LABORATORY EXPERIMENTS WITH TREATED BLOSSOM

Methods

Bunches of cut flowers were treated with several commercial preparations of D.D.T. and B.H.C., the sprays allowed to dry and a drop of honey placed in the centre of each flower.

Workers of *A. mellifera* collected from the hive were starved at room temperature for about 2 hr. until in a condition where walking movements were normal but flying was inhibited. They were then placed on the treated blossom in the laboratory for a period of 5 min., during which time they crawled actively from flower to flower consuming the droplets of honey. Bees on untreated blossom were used as controls.

The conditions were artificial in that the bees were chilled and starved with the result that their resistance was probably lower than normal. Moreover, by crawling

instead of flying from flower to flower they were in continuous contact with the insecticide but on the other hand a total period of 5 min. contact with the treated surface is less than would be expected under field conditions.

This technique was used to determine: (1) toxicity of D.D.T. and B.H.C. at different concentrations; (2) speed of toxic action of B.H.C. at different concentrations; (3) persistence of toxicity of B.H.C.

Description of experiments

(1) *Toxicity at different concentrations.* Three plant species were used, apple, cineraria and Michaelmas daisy. Bunches of open blossom were treated with commercial dusts and sprays of D.D.T. and B.H.C. Ten to thirty bees were placed on the flowers of each treatment and after 5 min. were caged and placed in the incubator. Table 6 shows the percentage kills on the following day.

TABLE 6. *Percentage kills of adult workers of* Apis mellifera *on the day following treatment with D.D.T. and B.H.C. on sprayed blossom*

Treatment	(% D.D.T.)	Apple (Worcester Pearmain)	Cineraria	Michaelmas daisy
Guesarol E spray	1	0	—	4
	0·5	—	0	4
	0·2	—	0	—
Guesarol dust	5 (% γ-isomer)	0	—	—
B.H.C. P530 spray	0·10	100	100	100
	0·052	52	69	80
	0·039	—	—	70
	0·026	0	0	100
	0·012	—	9	—
	0·0065	—	0	—
B.H.C. P.P. flea beetle dust	0·2	—	—	100
Control—untreated	—	0	0	0

The above experiment on apple blossom was repeated with males and females of the solitary bee *Andrena flavipes*. Kills caused by both insecticides were insignificant but the results are not comparable with those of the *Apis mellifera* experiments since the behaviour of the bees was entirely different. 5% D.D.T. dust and 1% D.D.T. spray were highly repellent, the bees refusing to remain on the flowers while on B.H.C.-treated blossom the bees were inactive and showed little desire to feed.

(2) *Speed of toxic action of B.H.C.* Bees caged after 5 min. contact with B.H.C.-treated blossom of Michaelmas daisy were placed in the incubator and careful examination made of their condition at 15 min. intervals. The following two stages in the development of toxic symptoms were determined.

(*a*) Slightly affected. Hyperactivity with some loss of co-ordination, the bees frequently falling on their backs and finding difficulty in righting themselves. This stage is important because it was felt that bees in this condition would be incapable

of returning to the hive with contaminated pollen and nectar. Under laboratory conditions mildly poisoned bees exhibited these symptoms but recovered completely after 1–2 days.

(*b*) Moribund. Only twitching movements of body and legs noticeable, a condition always followed by death.

Table 7 shows the lengths of time before slightly affected and moribund bees were noticed in the various treatments.

TABLE 7. *Speed of toxic action of B.H.C. on workers of* Apis mellifera, *showing the length of time after treatment before slightly affected and moribund individuals were observed*

Treatment	(% γ-isomer)	Slightly affected (min.)	Moribund (min.)	100% kill (min.)
B.H.C. P530 spray	0·21	15	30	180
	0·10	45	105	195
	0·052	60	135	—
	0·039	75	150	—
	0·026	75	180	—
B.H.C. P.P. flea beetle dust	0·2	60	135	—

(3) *Persistence of toxicity of B.H.C.* In this experiment branches of apple (Worcester Pearmain) and Michaelmas daisy blossom treated with B.H.C. P530 spray were kept in an open insectary protected from rain but exposed to other forms of weathering. Toxicity of the blossom on the day of spraying and on the 4 days following spraying was determined as previously described. Twenty to forty bees were used for each treatment.

Table 8 shows the percentage kills caused on successive days after spraying.

TABLE 8. *Percentage kills of adult workers of* Apis mellifera *caused by 1–4 days old films of B.H.C. P530 spray on open blossom*

	Apple				Michaelmas daisy	
Number of days after spraying	0·10% γ-isomer		0·052% γ-isomer		0·052% γ-isomer	
	Treatment	Control	Treatment	Control	Treatment	Control
0	100	0	63	0	79	15
1	57	0	64	0	57	15
2	—	—	44	0	40	5
3	33	0	—	—	27	0
4	—	—	18	0	3	10

Analysis of results

Results show that under the conditions of the experiment B.H.C. P530 spray on open blossom is toxic to *A. mellifera* workers when the γ-isomer content is greater than about 0·012%. At 0·052% γ-isomer, which is a high field concentration, there was an interval of 1 hr. after treatment before toxic symptoms were noticeable,

suggesting that the speed of toxic action of B.H.C. is not sufficiently high to incapacitate contaminated bees before they return to the hive.

Results of experiments on persistence of B.H.C. show that open blossom treated with this insecticide is liable to be toxic for at least 3 days.

Under the conditions of the experiment the D.D.T. preparations were non-toxic to *A. mellifera* and highly repellent to *Andrena flavipes* workers.

Experiments with bees foraging under natural conditions

Methods

The effect of certain commercial preparations of D.D.T. and B.H.C. on bees working treated crops under natural conditions was determined by two methods.

In the first method the effect of only short periods of contact was determined by catching and caging bees which had been working treated blossom for a known length of time. Bees working an untreated patch of blossom were similarly collected and used as controls. Kills were recorded on the following day.

The second method was designed to determine the toxicity of treated blossom to bees visiting it continuously over a period of from 1 to 6 days. This method takes advantage of the fact that a proportion of the foraging honey-bee population works the same group of plants for several days in succession (Butler, Jefree & Kalmus, 1943). A certain area of blossom was sprayed or dusted and all bees visiting the treated flowers counted and marked with a quick-drying paint (Butler *et al.* 1943). A different colour was used to mark control bees which visited an untreated area of blossom. By using several different colours on thorax and abdomen it was possible to distinguish bees that had been making regular daily visits to a particular area of blossom for a period of up to a week. The percentages of bees returning from day to day to the treated and untreated plots were compared and the insecticide considered toxic if the numbers of bees returning to the treated plots were significantly lower than the control. Otherwise it was concluded that the insecticide was non-toxic.

Three plant species, apple, white mustard, and cotoneaster were used in these experiments. Apple and mustard are worked by honey-bees for both nectar and pollen and cotoneaster for nectar alone.

Method I. Description of experiments

Apple blossom. Branches of trees in open blossom were treated with commercial sprays containing 0·5% D.D.T. and 0·013 and 0·052% γ-B.H.C.

It was observed that bees did not visit the sprayed blossom until it was dry. B.H.C. treatments were not repellent on apple or any other plant. Repellence was noticeable in the D.D.T. treatment and it was probably caused, not by the D.D.T. itself, but by the 10% of carrier material which damaged the anthers and caused shrivelling of the petals. A 0·2% D.D.T. spray containing 4% carrier and a 5% D.D.T. dust, used in subsequent experiments, were not repellent.

Bees which had been observed working the sprayed blossom for at least a minute

were caged and placed in an incubator as previously described. Control bees were collected from a branch of unsprayed blossom.

A branch with blossom in the pink bud stage was treated with B.H.C. spray containing 0·052% γ-isomer. Two days later the blossom opened and visiting bees were collected and caged as before.

Mustard blossom. A plot of flowering white mustard was heavily dusted with B.H.C. P.P. flea beetle dust containing 0·2% γ-isomer. Bees, after working for about a minute were collected from this and also from an untreated plot.

Table 9 shows the kills of adult worker bees on the first day after collection from the various treatments of apple and mustard. Death rate after the first day was not significantly higher than that of the controls.

TABLE 9. *First day kills of adult workers bees collected after working for a minute on blossom treated with commercial B.H.C. and D.D.T. preparations*

Blossom	Treatment	Number of bees per treatment	Percentage kill
Apple (open blossom)	Guesarol E spray, 0·5% D.D.T.	10	0
	B.H.C. P530 spray, 0·052% γ-isomer	14	64
	,, ,, 0·013% γ-isomer	22	27
	Untreated control	20	15
Apple (sprayed during pink bud stage)	B.H.C. P530 spray, 0·052% γ-isomer	10	0
	Untreated control	10	0
White mustard (open blossom)	B.H.C. P.P. flea beetle dust, 0·2% γ-isomer	30	17
	Untreated control	30	0

Several species of solitary bee (*Andrena* spp. *haemorrhoa*, *jacobi*, *varians*, and *armata* and *Osmia rufa*), visiting open apple blossom sprayed with 0·2% D.D.T. were collected and caged. None of these died during the following 9 days.

Method II. Description of experiments

Apple blossom. An apple tree (Newton Wonder) in open blossom was treated with Guesarol E spray containing 0·2% D.D.T. and another untreated tree (Bramley Seedling) 20–30 yards distant used as a control. One hundred bees were marked on each tree and bees returning on the two subsequent days counted and remarked. Table 10 shows the returns obtained. Blossom on the control tree was beginning to fall and this probably accounts for the low returns obtained.

Mustard. Plots 7 × 4 ft. were sown about 20 yards apart and used when in flower for marking experiments, one or two plots for each treatment. Bees, when visiting mustard, alight directly on the flowers and do not come into contact with the foliage, so, as the flowers last only 1–2 days, an insecticide would probably not affect bees for more than a few days after application and the same plots could be used for different experiments at 7–10-day intervals. Plots were heavily dusted with 5% D.D.T. Guesarol dust and 0·2% γ-B.H.C. P.P. flea beetle dust and untreated plots

used as controls. Counting and marking of bees was begun immediately after dusting. Three experiments were carried out, the results of which are shown in Table 10.

TABLE 10. *Daily returns of marked* Apis mellifera *workers to blossom treated with commercial D.D.T. and B.H.C. preparations*

Blossom	Treatment	Numbers counted and marked on first day	Numbers returning on subsequent days					Significance of difference
			Second	Third	Fourth	Fifth	Sixth	
Apple	Guesarol E spray, 0·2 % D.D.T.	100	27	10	—	—	—	Control unreliable
	Control	100	7	3	—	—	—	
White mustard	Guesarol dust, 5 % D.D.T.	51	6	3	1	—	—	$\chi^2 = 10{\cdot}13$. Highly significant
	B.H.C. P.P. flea beetle dust, 0·2 % γ-isomer	14	0	0	0	—	—	$\chi^2 = 8{\cdot}15$. Highly significant
	Control	45	18	10	7	—	—	
	Guesarol dust, 5 % D.D.T.	82	36	15	Marked bees collected and caged. No deaths for 6 days			$\chi^2 = 3{\cdot}99$. No significant difference
	Control	83	25	13				
	Guesarol dust, 5 % D.D.T.	30	11	—	—	—	—	$\chi^2 = 2{\cdot}94$. No significant difference
	B.H.C. P.P. flea beetle dust, 0·2 % γ-isomer	64	2	—	—	—	—	Data insufficient (returns too small)
	Control	38	7	—	—	—	—	
Cotoneaster horizontalis	Guesarol E spray, 0·5 % D.D.T.	84	62	41	30	13	11	$\chi^2 = 4{\cdot}09$. No significant difference
	Control	70	51	40	26	15	13	
	B.H.C. P530 spray, 0·013 % γ-isomer	96	Wet day no record	0	—	—	—	$\chi^2 = 63{\cdot}68$. Highly significant
	Control	44		24	—	—	—	

Cotoneaster. A bush of *Cotoneaster horizontalis* was treated with Guesarol E spray containing 0·5 % D.D.T. and an untreated bush 300 yards distant used as a control. Bees visiting the bushes were counted and marked for six consecutive days.

Flowers of cotoneaster being very small, a visiting bee must stand upon the leaves or stems while collecting nectar. Consequently, bees were in contact with D.D.T. on the foliage throughout the whole period of the experiment, although towards the end many of the flowers visited were those which had opened since spraying.

Cotoneaster bushes were also used to determine the toxicity of a B.H.C. spray. One bush was treated with P530 spray containing 0·013 % γ-isomer and bees counted and marked as before.

A few workers of *Bombus pratorum* L. visiting the cotoneaster bushes were likewise marked. These constantly visited the D.D.T.-sprayed bush but on the B.H.C. treatment were not observed after the day of spraying.

Analysis of results

Results in Table 9 show that under field conditions workers of *Apis mellifera* may be killed by contact for about a minute with open blossom treated with B.H.C. Furthermore, in marking experiments carried out over a period of days, returns of bees on B.H.C.-treated plots were always significantly lower than on the controls (Table 10) and it is concluded that the insecticide is highly toxic.

Experiments with D.D.T. showed no evidence of toxicity except in one case where there were low returns on a mustard plot treated with 5% D.D.T. dust. In two further experiments using 5% D.D.T. dust there was no evidence of toxicity, and where bees were caught and caged after visiting treated blossom for three successive days, no deaths were recorded during the following 6 days.

Some data on wild bees were obtained which suggest that B.H.C. is toxic and D.D.T. non-toxic to workers of *Bombus pratorum*. *Andrena* spp. and *Osmia rufa* were not affected by short periods of contact with D.D.T.-treated blossom.

Effect of D.D.T. on larvae of *Apis mellifera*

A number of preliminary experiments were carried out to determine the toxicity of D.D.T. to larvae of *A. mellifera*. Of these only one is worthy of mention, the results of which could not be confirmed by further experiment because brood production ceased unusually early owing to bad weather conditions.

Larvae are most likely to be affected by contaminated food carried back by workers to the hive and the following experiment was designed to determine whether this was possible. Workers were fed at the feeder of a hive with D.D.T. in syrup coloured with 0·003% basic fuchsin which enabled movement of contaminated syrup through the hive to be determined. When 0·002% D.D.T. was used 500 c.c. were consumed overnight and numerous coloured workers observed on the following day. An examination of the combs showed that coloured syrup had been deposited by workers in storage cells and a certain amount fed to the larvae, many of which were coloured but otherwise normal and healthy. In another hive about 400 c.c. of 0·02% D.D.T. in syrup were consumed and numbers of workers were dead or dying on the following day. The brood was all normal but had presumably received none of the D.D.T.-contaminated syrup since no stored syrup or coloured larvae were observed.

The above data might suggest that contaminated food presents no danger to larvae but considerably more experimental evidence is required particularly with regard to the effect of contaminated pollen.

Discussion

Results show that in the laboratory under rather drastic conditions B.H.C. as a contact insecticide is highly toxic to *Apis mellifera* workers, commercial preparations with γ-isomer content below field concentration causing 100% kill. Under the same conditions, field concentrations of D.D.T. are only partially toxic.

Stomach-poison action was determined using laboratory preparations which differed from the commercial products in both composition and particle size. Consequently, the results should be treated with reserve when stomach-poison action under field conditions is considered. The importance of particle size is shown by the fact that D.D.T. in colloidal form is about four times more toxic than a crystalline preparation. Lead arsenate under these conditions is less effective than D.D.T. while γ-B.H.C. by comparison is very highly toxic.

Laboratory experiments on contact poisoning with various bumble-bee species show that workers have a resistance to D.D.T. and B.H.C. comparable with that of *A. mellifera* workers. Queens and drones are more resistant, particularly to D.D.T. which at high concentrations and under drastic conditions has little effect on them. This is a fact of some importance since in spring when fruit blossom is sprayed the foraging *Bombus* population is represented almost entirely by queens and their destruction would entail the loss of up to 500 workers per queen later in the season.

In the field, experiments show that *Apis mellifera* and *Bombus* workers are apparently unaffected by visits to open blossom treated with commercial D.D.T. sprays and dusts. These results conflict with those of laboratory experiments where D.D.T. as a purely stomach poison was shown to be more toxic than lead arsenate, the use of which is a proved danger to honey-bees in the field. This leads to the conclusion that though, in the commercial preparations used, D.D.T. on open blossom is apparently safe to bees, there is always the danger that in a more activated form it may prove toxic, a fact which should be remembered when use on open blossom, or on a large scale is necessary.

Results of field experiments with B.H.C. confirm those obtained in the laboratory and show that commercial preparations on open blossom are lethal to bees. B.H.C. on open apple blossom may be harmful for at least 3 days after treatment and in view of the short period of contact required to kill, it is considered that sprayed foliage presents a danger to visiting bees by chance contact even when plants are sprayed before the blossom opens.

No repellence which could be attributed to the toxic principle in B.H.C. preparations was noticed, and it is concluded that the use of this insecticide on open blossom is a serious menace to the foraging bee population. Since the speed of toxic action of B.H.C. is apparently not sufficiently rapid to prevent contaminated workers returning to the hive there is the additional danger that nurse bees may be poisoned by contaminated pollen and nectar.

No experiments were carried out to determine the effect of B.H.C. on honey-bee larvae but preliminary work with D.D.T., which requires confirmation, suggests that this insecticide is not dangerous.

We should like to thank Dr F. Tattersfield and Dr C. G. Butler for their helpful suggestions and criticism and Mr A. H. McIntosh for advice regarding the laboratory preparations of certain insecticide suspensions.

REFERENCES

Butler, C. G., Finney, D. J. & Schiele, P. (1943). Experiments on the poisoning of honey-bees by insecticidal and fungicidal sprays used in orchards. *Ann. appl. Biol.* **30**, 143–50.

Butler, C. G., Jefree, E. P. & Kalmus, H. (1943). The behaviour of a population of honey-bees on an artificial and on a natural crop. *J. exp. Biol.* **20**, 65–73.

Cherian, M. C. & Mahadevan, V. (1946). Preliminary trials with D.D.T. and Gammexane against the Indian bee, *Apis indica*. *Indian Bee Journal*, **8**, 128–30.

Eckert, J. E. (1945). The effect of D.D.T. on honey-bees. *J. econ. Ent.* **38**, 369–74.

Filmar, R. S. & Smith, C. L. (1944). D.D.T. as a contact poison for honey-bees. *J. econ. Ent.* **37**, 537.

Frings, H. (1944). The loci of olfactory end-organs in the honey-bee, *Apis mellifera* Linn. *J. exp. Zool.* **97**, 123–34.

Holst, E. C. (1944). D.D.T. as a stomach and contact poison for honey-bees. *J. econ. Ent.* **37**, 159.

McIntosh, A. H. (1947). Relation between particle size and shape of insecticidal suspensions and their contact toxicity. I. D.D.T. suspensions against *Tribolium castaneum* Hb. *Ann. appl. Biol.* **34**, 586–610.

Weissmann, R. (1942). Ueber neue, wirksame Arsenersatztoffe im Obstbau und ihre Bedeutung für die Bienenzucht. *Schweiz. Bienen. Ztg.* **65**, 227–9.

(*Received* 18 *April* 1947)

THE PHYTOTOXICITY OF D.D.T. AND OF BENZENE HEXACHLORIDE

By ROSEMARY I. STOKER

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts

(With Plate 1)

Spraying with two preparations of D.D.T. had a negligible effect on the maturation of outdoor tomatoes and of summer cabbage. Spraying with a commercial D.D.T. suspension damaged cucurbits and treatment with the suspension base distorted glasshouse cucumbers during one season only.

Benzene hexachloride (B.H.C.) sprays did not affect the yield or flavour of tomatoes, but, at concentrations higher than those normally used, they severely scorched radish, turnip, swede, kale, spinach and beetroot seedlings. The application of B.H.C. dusts stunted seedlings of radish.

On one occasion young potato foliage was damaged by relatively high concentrations of two compounded B.H.C. sprays and by B.H.C. dust but late-season older foliage was unaffected. A spray compounded from B.H.C. dispersible powder caused tainting of potatoes, peas, carrots, beetroot, marrows, cauliflowers and lettuce. Apples and plums treated with B.H.C. spray preparations developed a taint after cooking.

A considerable amount of work has been carried out on the toxic action of D.D.T. and B.H.C. to plant pests and to beneficial insects, but relatively little is known about their effect on plant growth. In summarizing the work already carried out on the phytotoxicity of D.D.T., West & Campbell (1946) mention reports of injury to citrus trees, grape vine foliage, upland cotton, cowpea, peanut, soybean, tomato, velvet bean, cucumber and various herbaceous plants. On many of these plants, however, the effect of D.D.T. has not been clearly differentiated from the effects of the media in which it was applied.

No data have been published on the phytotoxicity of benzene hexachloride (B.H.C.) but the manufacturers have issued a warning that for 2 years following the use of B.H C. dusts for wireworm control, potatoes grown on the treated soil are liable to be tainted.

Rates of application of D.D.T. and B.H.C.

These were heavy for normal agricultural use, but were average for horticultural purposes where low-pressure spraying apparatus is used. Approximate rates were as follows:

Tomatoes (assuming 10,000 plants/acre)
Cabbages (assuming 10,000 plants/acre)
Potatoes (assuming rows 2½ ft. apart) } 300–350 gal./acre.

Seedling vegetables (assuming rows 2 ft. apart) 100–150 gal./acre.

A. Trials with D.D.T. (1:1-*bis*(*p*-chlorphenyl) 2:2:2-trichlorethane) during 1945

Detailed experiments were carried out with summer cabbages and outdoor tomatoes to examine both direct toxic effects and indirect effects on maturation. Smaller scale trials under glass included cucumbers, marrows and tomatoes, and preliminary observations were also made on the effect of D.D.T. on various other vegetable crops grown out of doors. Two spray preparations were employed: Guesarol E, a dispersible powder containing 5% D.D.T., as a suspension in water; and pure D.D.T. in a benzene-water emulsion, with cyclohexylamine dodecyl sulphate as emulsifying agent. In addition to unsprayed controls, treatments also included the spray media from which D.D.T. had been omitted. Concentrations of solid material are expressed throughout as % w/v; concentrations of benzene are expressed as % v/v. Spraying was carried out with a bucket pump sprayer.

(1) *Tomatoes.* The plants (variety Sutton's Harbinger) were randomized in blocks of four, twelve plants being used for each of the following treatments:

(*a*) Unsprayed control.
(*b*) 3·8% Guesarol E spray base in water.
(*c*) 0·68% benzene, 0·04% cyclohexylamine dodecyl sulphate in water.
(*d*) 0·2% D.D.T., 3·8% Guesarol E base in water.
(*e*) 0·2% D.D.T. emulsion containing 0·68% benzene and 0·04% cyclohexylamine dodecyl sulphate.

The plants were raised from seed sown on 26 March and finally grown in 14 in. pots. They were sprayed three times during the growing season on 26 June, 8 July and 27 August. Ripe fruits were weighed, counted and examined for tainting effects.

A heavy deposit remained on the plants after the application of Guesarol E and Guesarol E base, which only gradually disappeared over a period of 2–3 weeks; although white at first it quickly developed a yellow staining which remained for several days. The day following treatment the tips and edges of the flower petals developed a brown scorching which was rather more pronounced on the plants treated with Guesarol E. Flowers in bud, however, continued to open normally and there was no deterioration of fruit set. No damage was caused by the D.D.T. emulsion or its medium. The crop weights recorded and the average number of fruits per kilogram are shown in Table 1. Guesarol E, containing 0·2% D.D.T. appeared to lower the yield slightly; no significant differences were caused by the other treatments. The white deposit left by the suspension necessitated washing the fruit, but left no unpleasant flavour.

Small-scale trials were also carried out with glasshouse-grown tomatoes using variety Sutton's Harbinger. The plants were raised from seed sown on 26 March, and were potted on and treated in the same way as before. Six fruit trusses were allowed to develop on each plant. The spray treatments were as in the previous

experiment, but applications were made only on 23 May and 6 June. Two plants were used for each treatment, and were randomized along a single row on the glasshouse staging. Ripe fruit was weighed and examined for tainting effects.

Guesarol E and Guesarol E base produced symptoms identical with those observed after their application to outdoor tomatoes. No lasting damage was recorded. There was a slight decrease in yield from the plants sprayed with Guesarol E, which again suggests that this insecticide may have a slight toxic effect. The crop weights are shown in Table 1.

TABLE 1. *The effect of D.D.T. on outdoor and glasshouse-grown tomatoes*

	Outdoor tomatoes		Glasshouse tomatoes.
	Average wt. of fruit per plant in kg. (±0·07)	Average number of fruits per kg.	Average wt. of fruits per plant in kg.
Control	2·37	24·1	1·88
Guesarol E base	2·26	24·2	1·91
Emulsion base	2·31	24·4	1·76
Guesarol E≡0·2% D.D.T.	2·03	24·0	1·74
Benzene emulsion≡0·2% D.D.T.	2·38	25·4	1·91

During the summer of 1946 the detailed experiment with outdoor tomatoes was repeated, using only the Guesarol E, and Guesarol E base, and unsprayed controls (Table 6). The differences in crop weights were negligible, and it was therefore assumed from the 2 years trials that D.D.T. in both emulsion and suspension forms could be used with safety on tomato plants.

(2) *Cabbages.* Summer cabbages, variety Winningstadt, were used in a detailed trial to determine whether D.D.T. had any adverse effect on the hearting capacity of this crop. The plants were raised in drills in the open; they were sprayed once in the seedling stage as a precaution against flea beetle attack, the treatments being as follows: drill I, 0·2% D.D.T. as Guesarol E; drill II, 0·2% D.D.T. in benzene-water emulsion; drill III, unsprayed controls.

The experimental plot was planted up in early summer. The five treatments employed were the same as those employed in the trial with outdoor tomatoes; thirty-six plants were used for each treatment, and were randomized in plots of twelve. Plants from drill I were used to plant up the plots receiving Guesarol E and Guesarol E medium treatments; plants from drill II were used to plant up the plots receiving D.D.T. emulsion and emulsion medium treatments; and plants from drill III were used for the unsprayed plots. Three applications were made as follows: (*a*) 24 March, initial dipping after lifting from drills and before planting; (*b*) 12 July, spraying immediately prior to the onset of hearting; (*c*) 31 July, spraying after hearting commenced.

The plants were inspected after planting out, and after each spraying. An attack of cabbage aphis necessitated one spraying with nicotine, which was applied to all

plots alike. Attacks by cabbage caterpillars were kept down as far as possible by hand-picking of the larvae and destruction of egg batches. The plants were cut and weighed when fully hearted and the dates of cutting recorded.

No direct damage was recorded after the preliminary spraying of the seedling rows, but those treated with Guesarol E proved to be larger and stronger than the remainder of the plants.

Severe scorching was caused after the initial dipping by the emulsion base from which the D.D.T. had been omitted. The damage consisted of brittle white patches over the surface of the young leaves. Similar but less severe damage was caused by the D.D.T. emulsion itself. No damage was caused by the Guesarol E or its base, nor by subsequent applications of the emulsion.

Cabbage aphis was equally troublesome on all plots but defoliating caterpillars only caused damage to those plots which did not receive D.D.T. treatments. There was no significant difference in the time of hearting of the differently treated cabbages and only a negligible number failed to heart. The crop weights recorded are shown in Table 2.

TABLE 2. *The effect of D.D.T. treatments on the weight of hearted cabbages*

Treatments	Average wt. per cabbage in kg. (±0·11)
Control	2·12
Guesarol E base	2·27
Emulsion base	2·08
Guesarol E containing 0·2% D.D.T.	2·30
Benzene emulsion	2·34

D.D.T. appears to have no harmful effect on the hearting of cabbages at the concentrations employed. The slight increase in yield as compared with the control plots was probably due to beneficial insecticidal effect; if large numbers of caterpillars had not been hand-picked the differences in weight might have been much greater. The emulsion medium used was not satisfactory as it severely checked the young plants.

(3) *Cucumbers.* Following reports of the toxicity of D.D.T. to cucurbits (West & Campbell, 1946), preliminary small-scale trials were carried out during 1945 and 1946, on glasshouse cucumbers.

Plants of the variety Lockie's Perfection which had received their final potting into 14 in. pots were used. These were stood on the glasshouse staging and trained up parallel wires supported 6 in. away from the glasshouse roof. During the growing season the plants were fed at weekly intervals with standard liquid manure and were also top dressed with a compost of loam and manure.

The first trials were carried out during the early summer of 1945. Two plants were used for each of the following treatments: (*a*) 0·2% D.D.T. as Guesarol E; (*b*) 3·8% Guesarol E spray base; (*c*) unsprayed control.

Two sprayings were applied, the first when the plants were approximately 18 in. high, on 25 May, and the second on 30 June. Frequent inspections were made after each application.

The day following the first spraying with Guesarol E containing 0·2% D.D.T., the edges of the young leaves were scorched brown, and as growth continued this was followed by distortion and downward curling at the leaf edges. Fully developed leaves appeared normal for approximately 5 days after spraying and then developed a permanent interveinal yellowing (Pl. 1, figs. 1 and 2). The same symptoms were produced after the second spraying but were not so severe, as the young leaves did not show signs of scorching until 4 or 5 days after treatment. Growth remained sparse until 3 weeks after the second spraying after which the main stems increased in length considerably, eventually becoming taller than the controls. During the final stages of growth one plant developed a flattening of the main stem similar to the more severe distortion which occurred after treatment with the Guesarol E base as described below. Although the damaged foliage did not recover, and side-shoots

TABLE 3. *The effect of Guesarol E and Guesarol E spray base on glasshouse cucumbers*

Treatment	Effect on leaves	Effect on stems	Number of fruits (two plants)	Total length of main stems	Insect damage
0·2 % D.D.T. +3·8 % Guesarol E base	Scorching and downward curling of edges of young leaves. Interveinal yellowing of older leaves	Slight flattening of main stem of one plant at end of season. Single leaves and fruits at nodes	10	(i) 9 ft. (ii) 8 ft. 9 in.	Red spider present. Thrips absent on sprayed leaves
3·8 % Guesarol E base	Young leaves very slightly scorched. No curling or interveinal yellowing. Early death of basal leaves	Gradual and increasing flattening of main stems, producing broad nodes with profusion of growth	13	(i) 8 ft. (ii) 7 ft. 9 in.	Red spider and thrips present
Unsprayed controls	Normal	Normal. Single leaves and fruits at nodes	9	(i) 7 ft. 6 in. (ii) 7 ft. 6 in.	Red spider and thrips present

were considerably checked, the fruiting capacity of the plants did not appear to suffer. There was a noticeable absence of thrips damage, which occurred to a considerable extent on plants not receiving D.D.T. treatment. Red spider, however, was equally troublesome on all plants.

Treatment with the Guesarol E spray base produced a similar scorching of the young leaves, but this developed much more slowly and was less severe than that produced by the Guesarol E itself. No distortion of the leaves or interveinal yellowing occurred. Ten days after the first treatment the plants were making vigorous growth and appeared a darker green than the controls. At this period a gradual flattening of the main stems developed and grew progressively more pronounced as growth continued: it was accompanied by a profusion of growth at the growing tip and by broadened nodes bearing numerous leaves, tendrils and young fruits (Pl. 1, figs. 3 and 4). Very few side-shoots developed and the basal leaves died prematurely. A slight increase in fruiting resulted. The untreated plants developed normally—the main stems being rather thin and rounded, and single fruits and leaves developed at the nodes.

The results obtained are summarized in Table 3.

Two similar small-scale trials in the late summer of 1945 and 1946 confirmed the toxicity of D.D.T. in the form of Guesarol E to cucumber foliage, but the distortion produced by the spray base was not repeated, and it is thought that this may possibly have been caused by the presence of an impurity.

(4) *Vegetable marrows.* During the summer of 1945, a preliminary trial was carried out with bush-marrow plants. These were grown in 10 in. pots and were treated in the same way as the cucumbers, a single plant being employed for each treatment. Spray applications were made on 20 June and 18 July when the plants were in full growth.

Although no direct scorching was caused by the Guesarol E, interveinal yellowing and downward curling of the leaves appeared approximately 2 weeks after the first spraying, the symptoms increasing after the second spraying and causing stunted growth. No damage was caused to the plant sprayed with Guesarol E base, which was considerably more vigorous than the untreated plant. No fruits developed on any of the plants; this, however, was probably due to restricted root growth.

Further small-scale trials were made during the summer of 1946 on bush-marrows planted out in a prepared bed out of doors on 26 June. The same spray preparations were applied using two plants for each treatment. Only one application was made on 26 July.

Guesarol E again caused stunting and interveinal yellowing of the leaves. The base, however, appeared to have no abnormal effect when compared with the unsprayed controls, again indicating that the results obtained during 1945 may have been due to the presence of an impurity.

Other vegetable crops

A number of vegetable crops were grown under normal conditions out of doors, and during their growing season were treated with Guesarol E ≡ 0·2% D.D.T., and with spray base alone.

The results indicated that at the concentrations employed, D.D.T. has no direct toxic or abnormal effect on the following crops: lettuce, radish, turnip, pea, runner bean, french bean, broad bean and carrot.

B. Trials with benzene hexachloride (B.H.C.)* during 1946

In making preliminary observations on the phytotoxicity of B.H.C., direct and indirect toxic effects were examined, particular attention being given to its effect on the flavour of food crops. Trials were carried out on seedling and maturing vegetable crops, and on some fruit crops. Two commercial spray preparations were used throughout: Dispersible Powder in water, and Liquid Agrocide 3 in water: they

* The commercial B.H.C. used consisted of a mixture of isomers of benzene hexachloride, or more properly hexachlorocyclohexane. The γ-isomer has so far proved the most toxic insecticidally, but may not be responsible for any of the effects described here. The approximate proportions of the isomers in the preparations used are as follows: α 70%; β 5%; γ 10–12%; δ + small amounts of impurities, 13–15%.

are expressed below as % w/v, and % v/v of γ-isomer respectively. D034 General Purpose Dust and P.P. flea beetle dust were also used to a limited extent and are expressed as % w/w of γ-isomer. Seedling vegetables were sprayed with a bucket pump sprayer: later in the season a Mysto hand-sprayer was used. Dusts were applied by gentle shaking through a muslin bag.

1. *Seedling vegetables*

The following vegetable crops were used in trials to examine the effect of B.H.C. applications during the seedling stage: radish, turnip, swede, spinach, beetroot, pea, carrot and onion. The onions were raised under glass and transplanted; seeds of other vegetables were sown out of doors under normal conditions. Short lengths of drill were treated with each of the following preparations:

(*a*) Dispersible Powder $\equiv$ 0·2 % γ-isomer.

(*b*) Dispersible Powder $\equiv$ 0·04 % γ-isomer.

(*c*) General Purpose dust containing 0·5 % γ-isomer.

(*d*) P.P. flea beetle dust containing 0·25 % γ-isomer (applied to swedes and turnips only).

Single applications were made when the majority of seedlings were expanding their first true leaves.

Dispersible Powder $\equiv$ 0·2 % γ-isomer caused severe scorching of radishes, turnips, swedes, spinach and beetroot: the young leaves withered, and growing points were almost completely destroyed. Slight scorching occurred on peas, but no permanent harm resulted. Dispersible Powder $\equiv$ 0·04 % γ-isomer slightly scorched radishes and turnips, the latter being temporarily retarded also by the General Purpose dust.

Dispersible Powder at concentrations of 0·2, 0·1, 0·05, 0·01 and 0·005 % γ-isomer was sprayed on to newly germinated field kale seedlings in twelve randomized plots, each 8 × 15 ft.: the treatments thus included two replicates of each concentration, and two unsprayed plots.

As in the previous cases of seedling damage, the highest concentration of the Dispersible Powder containing 0·2 % γ-isomer caused severe scorching and withering. Only slight scorching in isolated patches was caused by the concentrations of 0·1 % γ-isomer and the lower concentrations did no damage to the seedlings.

A closer observation of the damage caused to seedlings by high γ-isomer concentrations was made in a small-scale pot experiment with glasshouse-grown radishes. Forty seeds were sown per 5 in. pot, and the spray applications were made 4 days after germination. The B.H.C. treatments consisted of Dispersible Powder $\equiv$ 0·2, 0·1 and 0·05 % γ-isomer; 0·2 % D.D.T. was included for purposes of comparison. Two replicates were provided for each treatment. The results are shown in Table 4 and are illustrated in Pl. 1, fig. 5.

The same procedure was followed in a trial to observe the effects of dusts on the germination and growth of radish seedlings. The treatments consisted of General Purpose dust containing 0·5% γ-isomer, P.P. flea beetle dust containing 0·2% γ-isomer and D.D.T. Guesarol dust containing 5% D.D.T. Forty seeds were sown in each pot, and two replicates provided for each treatment. The first application was made immediately after sowing and before covering the seed, and the second 4 days after germination. The dusts were distributed by means of a muslin bag. The results are shown in Table 5 and illustrated in Pl. 1, fig. 6. The differences in the numbers of seeds germinating are only barely significant.

TABLE 4. *A comparison of the effects of B.H.C. and D.D.T. on radish seedlings*

Treatment	Results
B.H.C. Dispersible Powder ≡ 0·2% γ-isomer	Severe yellowing and scorching especially round leaf edges and growing tips
B.H.C. Dispersible Powder ≡ 0·1% γ-isomer	Dwarfing and some scorching
B.H.C. Dispersible Powder ≡ 0·05% γ-isomer	Dwarfing and very little scorching
Guesarol E ≡ 0·2% D.D.T.	Seedlings normal though slightly smaller than controls
Unsprayed controls	Seedlings rather drawn

TABLE 5. *A comparison of the effects of B.H.C. and D.D.T. dusts*

Treatments	Number of seeds germinating per 40	Effects after germination	Effects after second treatment
B.H.C.	(i) 30	None	Slight dwarfing
General Purpose dust	(ii) 28		
P.P. flea beetle dust	(i) 26	None	Dwarfing and scorching of cotyledons
	(ii) 23		
D.D.T. Guesarol dust	(i) 34	None	None
	(ii) 31		

Effect on seedlings. It was concluded that at average field concentrations B.H.C. Dispersible Powder spray can be used with safety on a number of seedling crops, but that at concentrations above 0·1% γ-isomer severe scorching may be caused to brassicas, spinach and beetroot. The slight retarding effect resulting from the application of the General Purpose dust and the more pronounced stunting caused by the P.P. flea beetle dust to radish seedlings grown under glass, indicate that these should be applied with care in the field.

2. *Tomatoes*

A detailed experiment with B.H.C. on outdoor tomatoes (variety Sutton's Harbinger) was conducted in the same way as that dealing with the effect of D.D.T. The plants were raised from seed sown on 15 March and treated on 5 July and 9 August, when the plants were in full growth but before the fruit had started to

ripen. They were randomized in blocks of three, nine plants being used for each of the following treatments:

(*a*) Unsprayed controls.

(*b*) 3·8% Guesarol E spray base.

(*c*) Guesarol E ≡ 0·2% D.D.T.

(*d*) B.H.C. Dispersible Powder ≡ 0·01% γ-isomer.

(*e*) B.H.C. Liquid Agrocide 3 ≡ 0·015% γ-isomer.

(*f*) B.H.C. Liquid Agrocide 3 ≡ 0·0075% γ-isomer.

(*g*) B.H.C. Liquid Agrocide 3 ≡ 0·0037% γ-isomer.

The weights of ripe fruit at different periods through the summer were recorded and samples tested to determine tainting effects. Owing to poor weather conditions a very high proportion of the fruit failed to ripen and was picked green.

The results shown in Table 6 indicate that, at the concentrations employed, both B.H.C. preparations could be used with safety on outdoor tomato plants. No direct toxic effects were caused and the differences in the weight of fruit gathered and in the total amount of fruit ripening from the different treatments were not significant. At the first picking, ripe fruits gathered from plants which had previously been sprayed with Dispersible Powder at 0·01% γ-isomer, had an unpleasant flavour; this, however, was not noticed at any subsequent pickings.

TABLE 6. *The effect of B.H.C. and D.D.T. on outdoor tomato plants*

Treatment	Weights of fruit per plant in kg. ± 0·06	Proportion of crop ripening (%)
Unsprayed controls	1·30	20
3·8% Guesarol E base	1·25	22
Guesarol E ≡ 0·2% D.D.T. + 3·8% base	1·36	22
B.H.C. Dispersible Powder ≡ 0·01% γ-isomer	1·39	19
B.H.C. Liquid Agrocide 3 ≡ 0·015% γ-isomer	1·32	17
B.H.C. Liquid Agrocide 3 ≡ 0·0075% γ-isomer	1·47	18
B.H.C. Liquid Agrocide 3 ≡ 0·0037% γ-isomer	1·43	22

A small-scale trial was also carried out to determine the effect of B.H.C. applications on glasshouse tomatoes raised from seed sown on 15 March. Two plants were sprayed with each of the following Liquid Agrocide 3 concentrations: 0·015, 0·075, 0·005 and 0·0037% γ-isomer, applications being given on 28 June and 1 August. An additional plant was sprayed once with Dispersible Powder at 0·01% γ-isomer when the fruits were ripening. No direct toxic or tainting effects were caused by any of the treatments.

From these and the foregoing results it was concluded that B.H.C. can be used with safety on tomatoes both out of doors and under glass. Occasional tainting may possibly occur but the conditions causing this are not known.

3. *Potatoes*

In an experiment to determine the repellent action of various insecticides on aphides, potatoes, variety Majestic, were treated on 11 June with three B.H.C. preparations:

(*a*) Dispersible Powder ≡ 0·05 % γ-isomer.

(*b*) Liquid Agrocide 3 ≡ 0·01 % γ-isomer.

(*c*) General Purpose dust containing 0·5 % γ-isomer.

The weather conditions during and after treatment were dull with frequent showers, and the plants were young and tender and in active growth.

Severe scorching was caused by the Dispersible Powder spray; the leaves became hard and brittle, and were mottled with brown and yellow patches, some plants being damaged beyond recovery. Similar but less severe scorching was caused by the Liquid Agrocide 3 and General Purpose dust, and although temporarily checked, the plants subsequently recovered. In all cases, the growing points were less damaged than the more mature foliage.

As a result of this experiment it was decided to test both the Dispersible Powder and the Liquid Agrocide 3 sprays at a number of different concentrations on the same variety, Majestic. The plant foliage was slightly more mature than at the time of the previous applications. The following treatments were applied to short lengths of row on 17 June.

(*a*) Dispersible Powder ≡ 0·8, 0·4, 0·2, 0·1 and 0·05 % γ-isomer.

(*b*) Liquid Agrocide 3 ≡ 0·027, 0·01, 0·005 and 0·002 % γ-isomer.

None of the treatments caused any damage to the plants. During spraying and for 10 days after the weather was hot and sunny.

The trials were continued to determine whether damp weather conditions increased phytotoxicity.

Dispersible Powder spray at concentrations of 0·05, 0·01 and 0·005 % γ-isomer was applied to the variety Arran Banner on 16 July under damp misty conditions; a second application was made during unsettled weather on 24 July and was followed by heavy rain. No damage was caused on either occasion. Potatoes from the plots sprayed with Dispersible Powder at 0·05 % γ-isomer developed an unpleasant flavour which was very marked at harvesting, and still remained after 4 months' storage. The lower concentrations did not appear to have any marked effect. The varieties Majestic and King Edward received single applications of the following treatments on 28 August during dull windy weather: (*a*) Dispersible Powder ≡ 0·05 % γ-isomer; (*b*) Liquid Agrocide 3 ≡ 0·01 % γ-isomer; (*c*) General Purpose dust containing 0·5 % γ-isomer. No damage was caused by any of the treatments.

It was concluded from these investigations that under certain conditions high concentrations of B.H.C. in terms of γ-isomer, particularly as Dispersible Powder

spray, are liable to cause damage to young foliage. Concentrations of Dispersible Powder above 0·05% γ-isomer applied to foliage cause tainting of the crop.

4. *Other vegetable crops*

B.H.C. mainly in the form of Dispersible Powder was sprayed on to a number of vegetable crops, to test for any direct toxicity or tainting effects. The Mysto hand-sprayer used in all the tests gave a fine deposit; very little of the spray reached the soil and it is unlikely to have come into direct contact with the roots. After harvesting, the flavour of each crop was compared with that of an unsprayed control. No direct phytotoxic effect was observed on any of the treated crops.

Peas. Several plants were sprayed with Dispersible Powder at the following concentrations: 0·1, 0·05 and 0·01% γ-isomer. An application was made when the plants were in flower, and a second when the first pods were beginning to form. Tainting of the peas when cooked was quite distinct at the two higher concentrations, although there had been heavy rain during the period between spraying and harvesting.

Carrots. Carrot plants growing in drills received one application of Dispersible Powder at 0·2 and 0·04% γ-isomer and of the General Purpose dust. The plants were pulled during the summer before they were fully mature. Those treated with 0·2 and 0·04% γ-isomer, and with General Purpose dust had an unpleasant earthy flavour after cooking.

A further trial was carried out on carrots which were not harvested and sampled until late autumn. During the growing season two applications were made of Dispersible Powder at the following concentrations: 0·1, 0·05 and 0·01% γ-isomer. The highest concentration caused marked tainting which was discernible after cooking; the lower concentrations did not appear to have any effect.

Beetroot. Plants in full growth were treated in the same way as for peas, two applications being given. The sprayings were carried out in midsummer and the crop was not harvested until the autumn. Tainting of the cooked beetroot was marked at the highest concentration of 0·1% γ-isomer, though lower concentrations did not have any marked effect on the flavour.

Onions. The treatments were again as for peas—the crop being sprayed twice during the summer before bulbing and sampled during the autumn after a short period of storage. No tainting resulted from any of the treatments.

Lettuce. The plants were treated in the same way as for peas, before hearting had begun. Dispersible Powder at 0·1% γ-isomer caused a slightly bitter flavour, but lower concentrations had no effect.

Cauliflowers. Plants were sprayed once in full growth and a second time when nearing maturity, using Dispersible Powder at 0·05 and 0·01% γ-isomer. Both concentrations imparted a typical earthy flavour to the crop when cooked.

Radishes. Single applications of Dispersible Powder at 0·2 and 0·04% γ-isomer applied in the seedling stage caused no tainting to the crop.

Marrows. A range of concentrations of both Dispersible Powder and Liquid Agrocide were used on this crop. Single plants were sprayed once when the fruits were forming. The treatments were as follows: Dispersible Powder spray at 0·1 and 0·01% γ-isomer and Liquid Agrocide 3 at 0·015, 0·0075 and 0·005% γ-isomer. Heavy rain followed the treatments. Both preparations caused tainting of the cooked marrows at the concentrations used.

It was concluded from these results that the flavour of a number of vegetable crops is liable to be adversely affected by treatment with B.H.C. spray preparations. Of the crops tested the following were tainted: potatoes, peas, carrots, beetroot, marrows, cauliflowers and lettuce.

5. *Fruit crops*

Individual branches on trees of certain fruit varieties were sprayed with B.H.C. preparations during August when the fruits were well developed but not ripe. The following treatments were used: Dispersible Powder at 0·05% γ-isomer, and Liquid Agrocide 3 at 0·01% γ-isomer. The treated varieties were: Allington Pippin, Worcester Pearmain, and Newton Wonder apples, Victoria and Purple Pershore plums, and Conference pear. The fruits were sampled at harvesting time, excepting Newton Wonder apple and Conference pear, which were sampled after a period of storage.

Both preparations caused tainting of Victoria and Purple Pershore plums and Newton Wonder apples which were sampled after cooking.* The remaining dessert varieties were sampled without cooking and were not tainted.

These results obtained with only a few varieties indicate that B.H.C. has a tainting effect when sprayed on to developing fruit which is afterwards cooked.

Discussion

Both the synthetic insecticides D.D.T. and B.H.C. (benzene hexachloride) proved to be toxic to some of the plants used as test subjects in the trials.

The phytotoxicity of D.D.T. is not likely to prove a serious disadvantage, since, amongst the fairly wide range of test plants employed, only those belonging to the natural order Cucurbitaceae were adversely affected. Direct damage, however, can be caused by the use of unsatisfactory spray media; in addition, the use of spray suspensions leaving a thick unsightly residue on treated plants may result in loss of market value unless washing of the crop can be undertaken.

The application of B.H.C. on many plants was followed by a marked deterioration in the flavour of the crop; stunting and scorching of some seedling vegetables was also caused, and on one occasion potato foliage was damaged. The tainting was most marked on mild flavoured vegetables: the marrow, for instance, was particularly susceptible, while stronger flavoured crops such as onions and radishes were

* *Note added in proof.* F. C. Bishop (1946), *J. Econ. Ent.* **39**, 449–459, has reported that harvested fruit (apples) sprayed with B.H.C. had a musty flavour that rendered it unsaleable.

unaffected. Concentrations recommended for insect control in the field range from 0·04 to 0·025% γ-isomer, and in the majority of cases it would seem that both direct damage to seedlings and tainting of vegetables might be largely avoided by keeping within these limits. Tainting of fruit crops after cooking by 0·01% γ-isomer suggests that further work, particularly with culinary varieties is advisable before B.H.C. can be recommended for general orchard use.

My thanks are due to Dr F. Tattersfield for his helpful advice and criticism. I am indebted to Messrs Geigy and Co. of Manchester and to Imperial Chemical Industries for samples of commercial preparations of D.D.T. and B.H.C., and in addition have to thank Imperial Chemical Industries for information regarding the composition of B.H.C.

REFERENCE

WEST, T. F. & CAMPBELL, G. A. (1946). *D.D.T., the synthetic insecticide* (1st ed.). London: Chapman and Hall.

EXPLANATION OF PLATE 1

Fig. 1. A semi-mature cucumber leaf showing interveinal yellowing, and distortion of the tips due to scorching by Guesarol E ≡ 0·2% D.D.T.

Fig. 2. A semi-mature cucumber leaf from an unsprayed plant. The white speckling is due to thrips damage which was prevented on the plants sprayed with D.D.T.

Fig. 3. Growing point of cucumber plant sprayed with 3·8% Guesarol E base, showing flattening of main stem accompanied by abnormal profusion of growth.

Fig. 4. Growing point of unsprayed cucumber plant.

Fig. 5. (*left*) Radish seedling showing stunted growth and scorching due to the application of B.H.C. Dispersible Powder spray ≡ 0·1% γ-isomer. (*right*) Untreated radish seedlings.

Fig. 6. (*left*) Radish seedlings treated with P.P. Flea Beetle dust ≡ 0·2% γ-isomer showing stunted growth. (*middle*) Radish seedlings treated with Guesarol dust ≡ 5% D.D.T. (*right*) Untreated radish seedlings.

(*Received* 18 *April* 1947)

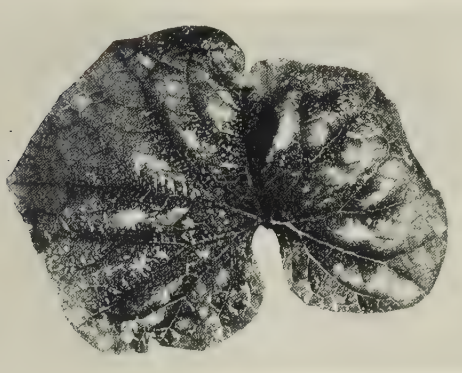


Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

TETRAZOLIUM SALT AS A SEED GERMINATION INDICATOR

By HELEN J. COTTRELL

Research Laboratories, Messrs May and Baker Ltd., Dagenham, Essex

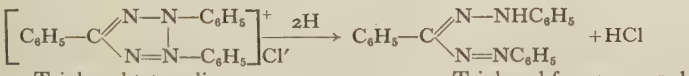
(With 2 Text-figures)

A preliminary assessment of 2:3:5-triphenyl tetrazolium chloride as a seed germination indicator has been made by testing wheat, barley, oats, peas and vetches. Results as reliable as actual germination tests have been obtained, and the optimum conditions for testing have been determined. The application of this technique to other seeds is also discussed.

The replacing of the standard laboratory test for germination by a chemical test which would yield results in a shorter time as well as being equally reliable has attracted attention in recent years. Various dyes such as methylene blue and compounds such as *o*-dinitrobenzene and sodium biselenite which are reduced phytochemically, have been used but the results proved unreliable. The chief criticism was that the chemical test could not distinguish between seeds that would produce normal and abnormal seedlings. An important advance was made by Lakon (1939) who evolved a topographical method for cereals using sodium biselenite, the objection here being the toxicity of the reagent.

The most recent developments have been made in Germany during the 1939–45 war (Hall, 1945) using the tetrazolium salts, Lakon (1942*a*, *b*) employing the 2:3:5-triphenyl tetrazolium chloride and 5-methyl-2:3-diphenyl tetrazolium chloride in his topographical method, particularly with maize. However, information in this country concerning the testing of other cereals and larger leguminous seeds is inadequate, so an assessment of this method using the triphenyl tetrazolium salt has now been made.

The tetrazolium salts have the advantage of being water-soluble, non-toxic, colourless and easily reduced to an insoluble red dye, the formazan, by chemical or phytochemical means.



Triphenyl tetrazolium chloride—colourless

Triphenyl formazan—red

Kuhn & Jerchel (1941) demonstrated that cultures of viable bacteria, fermenting yeast and germinating seeds are stained in the presence of the tetrazolium salts.

LAKON TECHNIQUE AND CLASSIFICATION

The Lakon technique consists of soaking the seeds in water overnight to start the chemical processes of germination, cutting the seeds longitudinally to bisect the embryo, and soaking the half seeds in the dark in a 1% solution for 3–4 hr. Then the seeds are examined individually and classified according to the parts of the embryo stained.

The classification is based on the fact observed by Lakon that in a monocotyledonous seed, normal germination will take place only if the whole embryo is still alive. Death of less than one-half of the scutellum may not prevent germination, however, for the conducting tissue might continue to function in transferring food from the endosperm to the seedling. Thus a seed in which the whole embryo except for half or less of the scutellum is stained, is considered capable of germination. Other variations in differential staining are observed also, as illustrated in Fig. 1.

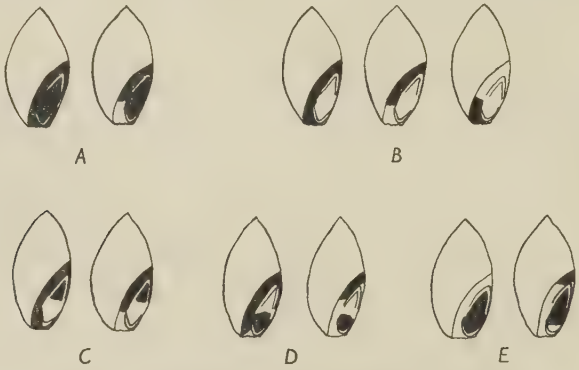


Fig. 1. Diagram of stained regions in cereals.

Only seeds labelled *A* are capable of normal germination, the *C* class will give seedlings without roots, and *D* will give seedlings without shoots. It is obvious that *B* and *E* types are incapable of germination although partially stained, while *C* and *D* are incapable of normal germination.

MODIFIED LAKON CLASSIFICATION FOR CEREALS

Results obtained with wheat, barley and oats were generally in good agreement with actual germination test results, but a few anomalies were observed, and it was evident that a finer distinction might be necessary. In maize the lateral roots arise from the first internode of the shoot, a characteristic feature not found in wheat, barley and oats. Lakon pointed out that so long as these adventitious roots were alive, it did not matter whether or not the main root developed; hence even though the root was unstained, staining of the region of the adventitious roots indicated that

normal germinations could take place as long as the scutellum and the rest of the shoot were stained.

In the other cereals, the secondary roots arise in the region immediately below the scutellar node, approximately where the embryo is attached to the scutellum, so that as long as this part of the embryo, as well as the shoot and scutellum are stained, a normal seedling would develop. Thus type *C* actually includes a class of seed which is capable of normal germination (*G*). The distinction between *C* and *G* is illustrated in Fig. 2.

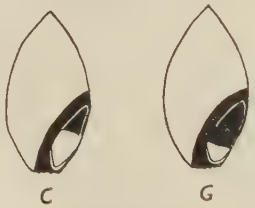


Fig. 2. Stained scutellar node in cereals.

Experimental procedure

Lakon technique

The seeds are selected at random, by the method of alternate halving if taken from a large sample, and steeped in tap-water overnight (18 hr.). They are then sectioned longitudinally through the embryo (peas and vetches can be prepared without removing the testas). Only one-half of each seed is used, and these halves are placed in a small Petri dish (7 cm.) which will hold about 100 grains or vetches, or twenty-five peas. A 1% tetrazolium salt solution is then poured over the seeds until they are all just immersed, and they are allowed to soak in the dark for 4 hr. at room temperature (*c.* 20° C.). By this time the viable seeds will be stained, and after washing with tap-water they are examined individually.

Preparation of tetrazolium solution

Since the tetrazolium salt is unstable in light, especially at higher temperatures, care must be taken in making a solution. The water is heated to a temperature not exceeding 60° C., and placed in an amber glass bottle with the solid salt. The mixture is shaken and the solid rapidly dissolves.

Germination tests

These were carried out by the standard method used at the Official Seed Testing Station, Cambridge. The seed-bed was washed silver sand, and conditions of time and temperature were as specified.

Comparisons of the results obtained by the standard germination test and by the tetrazolium test are set out in Table 1.

TABLE 1. *Comparison of germination and tetrazolium test results*

	Percentage of seeds capable of normal germination			
	Tetrazolium test			
Cereal	Number of seeds tested	Lakon classification	Modified Lakon classification	Standard germination test
Oats	3 × 100	85	—	88
Barley *A*	100	97	98	99
B	3 × 100	97	—	99
C	3 × 100	95	98	98
D	3 × 100	97	—	98
E	2 × 100	96	98	85
F	3 × 100	69	76	80
Wheat *A*	2 × 100	95	98	99
B	2 × 100	99	100	99
C	3 × 100	99	—	99
D	2 × 100	98	—	99
E	2 × 100	80	—	88
F	3 × 100	75	—	84
G	3 × 100	89	83	84
H	3 × 100	77	83	81
I	3 × 100	59	74	81
J	3 × 100	69	75	80
K	3 × 100	84	87	80
L	3 × 100	39	—	47
M	3 × 100	39	—	40
Maize *A*	90	65	72	70
B	50	76	76	75

Agreement between the two methods of determining germinative capacity is good in the majority of experiments, especially where the germination percentage is very high (95–99%). This, of course, is to be expected since there would not be the variation in types of staining to be found in poorer seed. An analysis of the numbers of stained seeds shows that in samples of grain with viability between 95 and 100% the largest number of partially stained types is in *C* and *G* (Fig. 2), i.e. a part of the root does not stain and the finer distinction between the areas stained certainly gives better agreement with germination tests. The next largest type in this group is where the scutellum only is stained.

In the viability group 80–90% (as determined by the test) there are three common types of partial staining, occurring almost to the same extent: (*a*) scutellum only stained, (*b*) part of the root unstained, and (*c*) whole embryo unstained. Again, the distinction between *C* and *G* types of staining gives more accurate results, although some are out of agreement. The only significantly different results are for a barley sample showing 85% germination but giving 98% viability by the tetrazolium test. An explanation is that the sample when tested by the standard laboratory method was not 'fully germination ripe', the grain being harvested under the abnormal 1946 conditions, and since several weeks elapsed before the tetrazolium test was done there was time for the sample to develop its full germinative capacity.

Generally, discrepancies between the two methods are such that the tetrazolium test gives lower results. This is especially noticeable in the wheat samples, differences varying from 4–8% at 80–90% germination. Only one example is 7% in excess of the germination results. This indicates that the test involves up to 10% experimental error, but the error in carrying out standard germination tests appears to be of this order. The schedule to the Seeds Regulations 1922 gives limits of variation for germination as below:

At or between 95–100	±4%	At or over 75 but less than 85	±8%
At or over 90 but less than 95	±6%	At or over 55 but less than 75	±9%
At or over 85 but less than 90	±7%	At or over 45 but less than 55	±10%

In this light, agreement between tetrazolium and germination tests is reasonable for seeds with 80–90% germination. Experiments giving 60–80% viability by the chemical test show that the largest group of abnormal seeds are those which are not stained at all, the next largest has the root partially unstained, closely followed by the group in which the scutellum is stained but the embryo is unstained. Thus, as the viability decreases the number of unstained seeds increases, as would be expected, but the percentage of the other types of staining remain more or less constant. This tendency is also noticed with the few samples in the lower germination group 30–60%, where the number of unstained grains is more than twice that of all the other partially stained seeds (Table 2).

TABLE 2. *Analysis of types of seed stained in tetrazolium experiments. Average percentage of partially stained grains*

Viability (%)	Embryo unstained	Root unstained (*C*+*G*)	(*G*)	Shoot unstained	Scutellum unstained	Whole seed unstained
95–100	<1	2	(1)	—	—	—
80–90	6	7	(2)	0·5	0·5	6
60–80	8	10	(7)	—	3	12
30–60	6	3	—	1	6	44

It was observed after 4 hr. soaking that in some samples of wheat the aleurone layer was stained in addition to the stained or partly stained embryos. Since the aleurone layer is composed of living cells, this is not surprising, but a superficial examination of the soaked grains which thus appear red all over would give a false impression of viability.

Two samples of wheat tested had been treated with an organomercuric fungicide which did not have any disturbing influence on the test.

Application to leguminous seeds

The same basic principles have also been applied to the testing of peas and vetches, the seeds being sectioned longitudinally through the embryo and between the two cotyledons. As one cotyledon was disregarded in the test, the only seeds capable of normal germination are those in which cotyledon, shoot and root are all stained. All

other types of staining have been observed, that is where one or two of these regions remained unstained. All were non-viable. In addition, 'hard seeds' which imbibe neither water nor stain were occasionally found.

Results are summarized in Table 3.

TABLE 3. *Comparison of germination and tetrazolium tests with leguminous seeds*

	Percentage of seeds capable of normal germination	
	Standard germination test	Tetrazolium test
Vetches	66	70
Peas	97	97

TABLE 4. *Effect of varying concentration of tetrazolium solution*

		Percentage capable of normal germination		
Seed	Concentrations (%)	Standard germination test	Tetrazolium test	Remarks
Barley	0·5	98	98	Intensity similar to 1 % staining
	1	98	97	—
	2	98	97	Dye precipitated on embryo
Wheat	0·5	40	39	Intensity similar to 1 % staining
	1	40	39	—
Peas	1	97	97	—
	2	97	93	Staining more even and intense than 1 %
Vetches	1	66	70	—
	2	66	67	Effect not as pronounced as peas

However, the number of tests carried out is not sufficient to show the accuracy of the method. There is a danger that in discarding one cotyledon a true result for viability might not be obtained, but on the other hand it is improbable that one cotyledon would be dead whilst the other remained alive. Again the question arises as to what extent the radicle must be stained, since a pea is still considered to have germinated normally if the main root dies provided that there are strong secondary roots. Examples of this kind were not discovered in the limited number of samples examined.

Since the cotyledons are so much larger and thicker than the cereal cotyledon, they are often unevenly coloured, sometimes just around the periphery, sometimes in a small area or streak on the inner surface, so that any stained region should be considered indicative of live tissue.

Determination of optimum conditions

The conditions of the test have been varied to obtain the optimum method of application. Concentrations of 0·5, 1 and 2 % were tested with barley, wheat, peas and vetches keeping the staining time at 4 hr. Results are recorded in Table 4.

The following conclusions can be made. Cereals require a 0·5 or 1% solution for even staining, a 2% solution causing precipitation of the dye on to the embryo, making detailed observations more difficult. Peas and vetches are stained more evenly and intensely with a 2% solution than with a 1% solution, consequently a 0·5% solution was not tried.

The period of staining also has been varied; 1 and 0·5% solutions were tested with wheat (germination 40%) for 2, 3 and 4 hr. (Table 5).

TABLE 5. *Effect of varying the duration of test*

Concentrations (%)	Time (hr.)	Viability	Remarks
0·5	2	40	Many very faintly or unevenly stained
0·5	3	42	Seeds unevenly stained
0·5	4	42	Staining compares with 1% solution at 4 hr.
1	2	39	Staining compares with 1% solution at 4 hr.
1	4	39	—

The result of these experiments shows that 2 hr. soaking in a 1% solution produces a good even stain comparable to that obtained after 4 hr., in other words reaction was complete, for this sample, within 2 hr. On the other hand if the concentration was halved, the time had to be doubled. Owing to the variation between samples it is desirable to allow a margin for the seeds that are more slowly stained, otherwise it is difficult to decide whether or not very lightly stained seeds are to be included in the counts.

No experiments were done varying the soaking time of peas and vetches as owing to the thickness of the cotyledons the penetration of the solution into the tissue would take longer and hence any time less than 4 hr. would be insufficient.

Limiting factors of the test

Extension of this method to other seeds is governed chiefly by their size; the embryos in the common cereal seeds are large enough to be observed conveniently, but with grasses the manipulative difficulties are great. Dicotyledonous seeds such as beans, peas and vetches and perhaps clovers would probably be suitable, but the smaller seeds like mustard, turnip and parsnip do not allow of really detailed examination, especially where the embryo is very small as in parsnip or folded up as in mustard.

Lakon (1942*b*), in his paper on maize, states that the stained seeds can be kept for 24. hr without change in the differential staining, but experiments with wheat and barley have shown that 18 hr. after staining the starch had begun to be hydrolysed, and moreover, the residual solution in the cells was reduced causing deposition of dye on the parts of the embryo. He also stressed that pre-soaking should be no longer than 18 hr. which is long enough to allow water to penetrate the tissues and so start the

chemical processes of germination without showing signs of sprouting. The tetrazolium solution is reduced immediately on contact with a reductase enzyme system, hence the time of staining depends on the rate of diffusion of the solution.

CONCLUSIONS

The time taken by the test is therefore about 24 hr., since it is advisable to examine the seeds within 2 hr. of becoming stained. In contrast, the standard germination test takes 10 days or more.

Although the time is reduced, the technique involves more tedious and exacting work in preparing and examining seeds, particularly in oats, since the caryopses have to be removed from the pales before they can be sectioned.

However, adoption of this method as a routine test where speedy evaluation of germination is important is quite possible since no special apparatus or botanical skill is required once the details have been mastered.

The latent causes of abnormal germination or failure to germinate may also be discerned by this method which would be especially valuable when used as a screening test. Extension to other smaller seeds cannot be envisaged, but it is probable that application to the larger leguminous seeds will produce promising results.

ADDENDUM

Since the preparation of this paper, Porter, Durrell & Romm (1947) in U.S.A. have published results of testing certain cereal and leguminous seeds by the tetrazolium method. For barley, oats, wheat, rice and buckwheat they maintained as a criterion for viability that the whole of the embryo and scutellum should be stained. This method, however, gave several discrepancies of 11–17% with oats and barley. Their results with rice are difficult to evaluate since three methods of germination gave varying results. For maize, Lakon's modified criteria for viability were accepted, a fact which produced results in good agreement with laboratory germinations. This supports the view expressed by the author of this paper that the detailed examination of the stained embryo is necessary for accurate results, and it is possible that had this been done the discrepancies with oats and barley would not have occurred. The results of Porter *et al.* (1947) agree with the conclusion above that a 2% solution is too strong but there is little difference between a 0·5 and 1% solution. The staining time in their experiments varied between 2 and 5 hr. Leguminous seeds tested were peas, soya beans and vetches but the staining time was 15–30 min. only. They draw the conclusion that the method is not entirely satisfactory since no indication of the condition of the epicotyl is given by the test. This is obviously another obstacle to applying the tetrazolium test to dicotyledonous seeds, besides the one mentioned in this paper, but more work needs to be done before any sound opinion can be expressed. Porter has also reported the deposition of the stain on the flat surface of the cotyledons, which was not observed here, but may be explained by

the age of the seeds tested, the peas and vetches being a year old while Porter does not indicate the age of the seeds used.

The author would like to express gratitude to Mr C. C. Brett of the Official Seed Testing Station and to Dr Wain of Wye College, for their advice, and to the Directors of May and Baker Ltd., for permission to publish this communication.

REFERENCES

Hall, S. A. (1945). *C.I.O.S. Rep.* xxvi, 73.
Kuhn, R. & Jerchel, D. (1941). *Ber. dtsch. chem. Ges.* **74**B, 941.
Lakon, G. (1939). *Ber. dtsch. bot. Ges.* **57**, 191.
Lakon, G. (1942*a*). *Ber. dtsch. bot. Ges.* **60**, 299.
Lakon, G. (1942*b*). *Ber. dtsch. bot. Ges.* **60**, 434.
Porter, R. H., Durrell, M. & Romm, H. J. (1947). *Plant Physiol.* **22**, 149.

(*Received* 12 *July* 1947)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 7 November 1947, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

The following paper was read and discussed:

The biology of earthworms and their effects on the soil. By Dr A. C. Evans.

Ordinary Meeting of the Association held on Friday, 5 December 1947, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

The following papers were read and discussed:

1. Oil seed crops in Great Britain. By Mr E. S. Bunting.
2. Control of weeds in oil seed crops. By Mr K. Holly.
3. Insect pests of *Brassica* seed crops. By Mr S. G. Jary and Mr K. Carpenter.
4. The mosquito eradication campaign in Sardinia.* By Dr J. R. Busvine.

OIL SEED CROPS IN GREAT BRITAIN

By E. S. Bunting, *Department of Agriculture, Oxford University*

Since 1941 many oil seed crops have been examined in the research programme under the general direction of Prof. G. E. Blackman. The most promising results have been obtained with sunflower (*Helianthus annuus*), oil poppy (*Papaver somniferum*) and linseed (*Linum usitatissimum*).

Of the various sunflower varieties tested Pole Star and Jupiter, selections from Russian varieties, have proved most suitable for this country. These 'semi-dwarf' varieties grow to a height of 4–6 ft.; they mature in approximately four months and the seeds have an oil content of 33–37 % dry weight. The Argentinian variety, Klein, will give seed yields equal to the Russian varieties but it is at least a month later, much taller, and has slightly lower oil and protein contents. In view of the length of the growing period of all varieties so far tested, early sowing is essential. Experimental evidence suggests that sunflower seedlings have considerable frost resistance and can be sown at the end of March; mid-April is probably the latest desirable sowing date.

Sunflowers show little response to fertilizer treatment. On average soils neither seed yield nor seed composition is significantly influenced by nitrogen, phosphorus and potassium.

Varieties respond differentially to variations in plant density. At densities of 4 plants/sq.yd. or less, the taller varieties such as Klein outyield the semi-dwarf varieties, but with increasing plant density the difference in yield per acre becomes progressively less. From 8 to 10 plants/sq.yd. is the optimum density for Jupiter and Pole Star. At such densities their seed yield does not differ significantly from that of Klein. The protein content of the seed is rarely affected by spacing changes, but the oil content is significantly increased with increased plant density. Here the explanation lies in the fact that the ratio of kernel to husk is inversely

* See Busvine, J. R. (1948). Recent mosquito eradication campaigns. *Nature, Lond.*, **161**, 189.

related to seed size, and since small seeds are associated with small heads, the oil content of closely planted crops is higher.

Studies in the factors affecting seed development during the ripening phase have shown that the changes in 'kernel' oil and protein weight give similar sigmoid curves—which differ from those of 'husk' weight. Partial defoliation does not affect seed development, but development is arrested by severance of the capitulum from the stem or the stem from the root.

Experimental work with oil poppy began in 1946, on a number of continental varieties. Peragis, a short-stemmed variety, is earlier maturing than the taller Mahndorfer. Both ripened satisfactorily in 1946 and 1947 and have given, in experimental plots, high seed and oil yields—up to 19 cwt./acre and 49% oil. Spacing trials suggest that the optimum plant density is 30–36 plants/sq.yd. The varieties have closed capsules and there is no seed loss on harvesting. With the ordinary farm machinery difficulties have yet to be overcome: the 1000 seed weight is only 0·5 g., very low seed rates are therefore necessary, the depth of sowing must be shallow and the germination may be erratic, with attendant weed problems.

The factors governing successful linseed cultivation have been extensively investigated during the past seven years. Comparative variety trials have proved that the recently bred American and Canadian varieties are superior to La Plata, the variety grown in this country during the first world war. Royal, Bison and Redwing are taller and less branched than La Plata. They are also earlier, and more even in ripening, easier to harvest and give greater yields. In seed composition Royal and Bison differ but slightly from La Plata, all three varieties having an oil content of 40–43% of the seed dry weight and a protein content of 23–26%. Redwing, a smaller seeded variety, has a slightly lower oil content (38–40%), but as it is the earliest variety so far tested, it should prove particularly valuable for Northern England and Scotland.

From development data on plants grown at a range of densities it has been found that seed yield per unit area is highly correlated with capsule production. Varying density has little effect on seed size or the number of seeds per capsule. At low densities, the formation of axillary branches is encouraged.

Linseed does not demand a high mineral nutrition level in the soil. Complete manuring has given an average yield increase of 8%. Factorial experiments show that the effects of phosphorus and potassium are small, but that there is often a significant response to nitrogen due almost entirely to an increase in the number of capsules per stem.

The effects on seed yield of different sowing rates and drilling methods have been studied: when the distance between the drills is kept constant at 6–7 in., 60 lb./acre is sufficient seed to give the optimum plant population. If the drill width can be decreased to 4 in., better seed yields are obtained with a sowing rate of 80 lb./acre. There is slight evidence that when 80 lb./acre is cross-drilled in 6 in. rows, the yield is higher than when the same amount of seed is sown 'one way'.

These investigations of linseed, oil poppy and sunflower crops in Great Britain, suggest that carefully chosen varieties are capable of giving yields of high quality seed which compare very favourably with those obtained in other countries.

CONTROL OF WEEDS IN OIL SEED CROPS

By K. Holly, *Department of Agriculture, Oxford University*

Weeds in sunflowers and oil poppies can be controlled by orthodox mechanical methods, principally inter-row cultivation. The problem in linseed is more difficult since a narrow drill width is necessary for maximum yield and the crop is very susceptible to weed competition. Therefore a technique of chemical weed control would be of considerable value in the cultivation of this crop.

Initial experiments on flax during 1943–5 indicated that treatment with sodium 2-methyl-4-chloro-phenoxyacetate (M.C.P.A.), one of the synthetic growth substances, at herbicidal concentrations caused no reduction in seed yield. Preliminary field trials in 1946 verified that this compound applied to linseed at 0·2 % concentration at 100 gal./acre caused no yield reduction, but killed a large number of annual weeds. Accordingly a detailed study of the effect of this type of compound on linseed has been carried out.

The crop is resistant and may be sprayed with safety when over 3–4 in. in height. In addition to M.C.P.A. various forms of 2:4-dichloro-phenoxyacetic acid (D.C.P.A.) are available for use as herbicides. Although both compounds are approximately equivalent in their killing effect on most annual weeds, the safety margin with regard to linseed seed production is greater with M.C.P.A. than D.C.P.A. Thus a 0·2 % solution of the former is perfectly safe, while with the latter, in the form of the acid or its sodium salt, a concentration of 0·1 % should not be exceeded. The ethyl ester of D.C.P.A. in oil-water emulsion is highly toxic to linseed.

At high concentrations, M.C.P.A. and D.C.P.A. as the sodium salts both cause similar reductions in the 1000 seed weight, while at the same concentrations D.C.P.A. brings about a much greater reduction in seed yield. Thus D.C.P.A. has a considerably greater effect than M.C.P.A. in reducing the number of seeds produced. Under some circumstances a very small, but statistically significant reduction in oil content of the seed may occur, and here M.C.P.A. and D.C.P.A. are similar in their action. On the basis of this evidence it is tentatively suggested that there are at least two effects of these compounds on the linseed plant, one on the metabolism concerned in seed production, the other on the initiation and morphological development of the flowers and seeds. With regard to the metabolic effect, the two compounds tested are identical, while the substitution of the methyl group by a second chlorine atom differentially affects the morphological development of the flowers and seeds.

As a result of this work a suitable technique for chemical weed control in linseed can now be put forward, using 0·2 % M.C.P.A. or 0·1 % D.C.P.A. at 100 gal./acre. Earlier work has demonstrated the efficiency of such solutions for killing a large range of annual weed species. With the elimination of weed competition by this method large increases in yield have been obtained.

INSECT PESTS OF *BRASSICA* SEED CROPS IN ROMNEY MARSH, KENT

By S. G. Jary and K. Carpenter, *N.A.A.S. Sub-centre, Wye, Kent.*

In the Romney Marsh area, 3-months-old swede plants (stecklings) are planted out in November at 15 × 24 in. In April the stecklings send up a flowering shoot which is topped at 6 in. to produce six to eight laterals. Secondary and tertiary branching follows later and the branches of adjacent plants eventually become interlaced. Flowering starts about mid-May and continues for several weeks. The pods mature towards late July and must be harvested before becoming overripe.

The three important insect pests associated with loss of seed are *Meligethes aeneus* (blossom or pollen beetle: locally known as 'flea'), *Ceuthorrhynchus assimilis* (swede seed weevil), and *Dasyneura brassicae* (bladder-pod midge).

Meligethes aeneus hibernates in the adult stage in rough herbage, migrating from mid-April onward to the crops as the first flower buds appear. Beetles continue to arrive until about mid-May, when the numbers reach a maximum, but the movement of beetles may be interrupted by a spell of cool, sunless weather, giving the impression that there are two separate infestations and sometimes a partial third. Feeding upon the unopened flower buds, these beetles destroy up to 50 % of the actual buds in severe attacks, with a corresponding reduction in the number of pods eventually formed. Eggs are laid in some buds, where the larvae feed upon pollen. Pupation takes place in the soil, a new generation of the beetles emerging from the latter part of June onwards. No material damage seems to result from the larval feeding and the important injury is that caused to the buds by the adult beetles. When the beetle

population exceeds about 30–50 per plant, considerable loss may result, but up to 200 beetles per plant is not unusual.

Ceuthorrhynchus assimilis occurs in company with *Meligethes aeneus* on the flower buds, but in much smaller numbers, seldom exceeding 10 per plant. Some injury to buds results, but the weevils are rather later in arriving, being most numerous as pods begin to form. They feed by puncturing young green pods and also lay eggs within them, the larvae feeding upon the immature seeds. Direct loss caused in this way is believed, however, to be comparatively small.

Dasyneura brassicae appears when flowers open and there are almost certainly several generations of these flies during the summer. Eggs are laid in the pods, where the numerous white larvae are readily found. Affected pods develop faster than normal, becoming rather swollen, and somewhat distorted. They usually contain some good seed, but split prematurely and shed the seed before harvest. Up to 25 % of pods may be affected.

It seems quite certain, as suggested by some continental workers, that *D. brassicae* is almost if not entirely dependent upon *Ceuthorrhynchus assimilis* for making punctures through which eggs of the former can be laid. *C. assimilis* thus assumes a further importance and the control of injury caused by *Dasyneura brassicae* may be intimately linked with control of the weevil.

Control. In 1941 a firm of contractors undertook the control of *Meligethes aeneus* on a field scale, using a *Lonchocarpus* dust of about 1 % rotenone content and later a dust containing benzene hexachloride. In Romney Marsh, although in general a high degree of control of *Meligethes aeneus* was obtained and in some crops heavy loss of buds avoided, the ultimate yield of seed was not always satisfactory. Moreover, until *M. aeneus* was controlled, the loss caused by *Dasyneura brassicae* could not readily be assessed and it was decided to carry out a detailed investigation.

Insecticides. Field and laboratory tests of insecticides showed that at the concentrations in use, rotenone, D.D.T. and benzene hexachloride were all lethal to *Meligethes aeneus* but less satisfactory against *Ceuthorrhynchus assimilis*. After it had been demonstrated that *Dasyneura brassicae* regularly utilizes the punctures made by *Ceuthorrhynchus assimilis*, for oviposition, it became clear that an insecticide of higher toxicity toward the weevil was desirable. The contractors concerned produced a dust containing pyrethrum which in 1944 gave very promising results. The difficulty still remained that even if a crop was dusted twice, the second application might be too early to give the maximum protection against the weevil. The interlacing of the plants prevented the passage of machinery through the field after the crop had reached a certain stage and so determined the latest time at which insecticides could be applied.

Time of application in relation to infestation. Tests of insecticides, combined with field observations, suggested that the apparent reinfestations of crops which occurred after treatment in some instances, were not due to failure to kill the insects. Studies in the build-up of populations made between 1944 and 1947 established a correlation between insect movement and weather conditions, leading to the discovery that beetle movement continued from about mid-April to mid-May, but ceased during cold periods. Crops treated and cleared just before a cold spell are therefore liable to reinfestation on the next warm day, a phenomenon frequently recorded. Provided therefore the initial infestation was not excessive, it was obviously an advantage to defer treatment until the majority of beetles had arrived. Moreover, in the earlier stages of growth, only a small percentage of the final number of flower buds was present on the plants and the loss of some proportion was not likely seriously to impair the final yield. It thus became customary to defer treatment until about the second week of May, depending upon the extent of infestation and weather conditions. If two treatments were to be given, the second was normally advised at the latest date when machinery could be taken through the crop. This gave the greatest probability of controlling *Ceuthorrhynchus assimilis* and thus, indirectly, the attack by *Dasyneura brassicae*. *Ceuthorrhynchus*

assimilis seemed to be rather more sensitive than *Meligethes aeneus* to weather conditions and reached the peak of population several days later. The full programme put into operation in 1945 was therefore to control *M. aeneus* by any suitable dust during the second week of May and to apply a dust containing pyrethrum 10–14 days later, to control *Ceuthorrhynchus assimilis* together with any *Meligethes aeneus* which had arrived in the meantime.

Sampling methods. Controlled field experiments were impracticable in the circumstances and, in any case, no methods were known by which brassica seed crops could be sampled for assessment of yield. With the most helpful co-operation of the Statistics Department of Rothamsted Experimental Station, attempts were made to obtain reliable data on the actual losses caused by the various pests and the degree of protection resulting from treatments. This involved a study of the development of seed-bearing branches, flowers and pods, on which subject a very large amount of information was obtained. It showed that the ultimate yield of seed is profoundly influenced by factors other than the intensity of insect attack, though it was not possible to evaluate these factors further. They are probably related to fertility conditions and are to some extent of a varietal nature. The nutrition of most seed crops has scarcely been explored in this country and it seems certain that the control of pests is by no means the only important factor.

REVIEWS

Decay of Timber and its Prevention. By K. St G. Cartwright and W. P. K. Findlay. Pp. vi + 294. Forest Products Research Laboratory. London: His Majesty's Stationery Office. 1946. 12*s.* 6*d.*

In publishing this work, the authors have no necessity to make the almost customary apology for adding yet another book to an already well-covered and even over-crowded field. On the contrary, this is the first complete book to be published which deals specifically with the decay of British timbers. The eminence of the authors, as instanced by their comprehensive and detailed knowledge of the subject, their long and varied experience, and their previous publications in this field, give this book a standing which places it high in the list of recent scientific publications.

The first four chapters, occupying some fifty pages, deal respectively with: the causes of decay of timber in general, laboratory technique for examining decayed timber, studies in the physiology of the wood-destroying fungi, and the effect of fungal decay on the physical properties, appearance, etc., of the wood. The next hundred pages deal with the particular decays of standing timber, Chapter V with the decays of conifers and Chapter VI with the decays of broad-leaved trees. This section, which occupies about one-third of the book, is most comprehensive. It includes diagnostic keys for identification of the fungi causing rots of conifers and of ash, beech, elm, oak and willow. These keys are based on a sequence of observations, from the position and appearance of the rot to the microscopic details of the rot and finally to the cultural characteristics of the isolated fungus. The use of the keys should enable at least some thirty of the commoner fungus pathogens to be determined with certainty. Accompanying the keys are detailed accounts of each of the fungi mentioned. Each detailed account gives the correct name of the fungus and the synonyms, and goes on to discuss its occurrence and to describe the appearance of the sporophores and their position on the tree. This is followed by a description of the gross characters of the decay and the microscopic characters of the fungus in the timber. The appearance of the fungus in culture, the growth, colour, and characters of the colony and any significant physiological factors are dealt with, and, finally, there is a statement as to its economic importance. The details of each fungus, in fact, amplify and confirm the brief indications given by the keys. Fungi of less economic significance are also briefly referred to. Chapters VII and VIII deal in the same extensive manner with the diseases of felled timber, worked timber in the open, and worked timber in buildings. The treatment here follows the same lines as above, with similar diagnostic keys and detailed accounts of the fungi, and naturally includes a very useful section on the dry-rot fungus, *Merulius lacrymans*. Chapter IX deals in a most interesting manner with methods of prevention of decays of felled timber in storage and shipment, while Chapter X deals with decays of timber in various uses. This ranges over such applications as buildings, mining, public and private vehicles, aircraft, shipping, sleepers, fencing and even garden stakes. Chapter XI considers the deterioration of manufactured wood products such as plywood, wood-pulp, wall-boards, etc. The remaining three chapters deal with durability, preservation and staining and discoloration of timber.

There is a very extensive bibliography, the references being conveniently placed at the end of each chapter. This, of course, has advantages and disadvantages, but perhaps, on the whole, the former outweigh the latter. The work is well-indexed and, an important point, the references to pages indicating the major treatment of the subject are in heavy type. A feature which increases enormously the value and interest of the book is some hundred excellent photographic illustrations and eight text-figures. In the photographs, some indication of

relative size is always given, and sometimes a scale is included. One of the most valuable features of the book is the combination of scientific investigation and practical application in all essential aspects of timber decay. It should appeal to a public extending far beyond the bounds of academic scientists. It is, in fact, an essentially practical treatment of an essentially practical subject.

The above outline does inadequate justice to the excellence of the book. It is a reviewer's duty to allot praise and blame in due proportion, but in this volume, such points as could be criticized refer to minor details which do not reduce a whole-hearted admiration for the work. At any time this book would have been appreciated, but coming as it does at a time when the timber shortage has fixed attention on the subject to an unusual extent, it is especially welcome.

W. H. WILKINS

Hormones and Horticulture: The use of special chemicals in the control of plant growth. By GEORGE S. AVERY, Jr. and E. B. JOHNSON. Pp. 326. New York and London: McGraw-Hill Book Co. Inc. 1947. 27*s*.

There are few branches of applied botany which have made such rapid and spectacular advances during the past decade as that dealing with plant hormones and growth regulating substances, and the appearance of an up-to-date text-book on the subject is a timely and welcome event.

The name of the chief author should, in itself, be sufficient recommendation for the book, for Prof. Avery is one of the leading workers in the field of plant hormone research, and his translation and revision of Boysen Jensen's *Wuchsstofftheorie*, published in this series of books in 1936, has for long been regarded as the standard text-book on the fundamental aspects of the subject. The present volume, on the other hand, will be of special value to those concerned with the practical application of growth substances in horticulture.

The title, in spite of its alliterative merits, perhaps fails to do justice to the extensive scope of the subject-matter, for, in addition to chapters dealing with hormones in relation to rooting, pre-harvest drop control, fruit-set, dormancy, weed control and other topics, there is much information on related subjects, such as the use of ethylene compounds for breaking dormancy, blossom-thinning sprays, and the production of polyploids by the use of colchicine.

The book contains 743 references to original papers in technical journals, and horticultural workers throughout the world owe a deep debt of gratitude to the authors for the excellent and impartial way in which they have summarized and evaluated such a large mass of data, some of which, such as that dealing with the hormone treatment of seeds, is still highly controversial. The only important omission seems to be the absence of any reference to the use of ethylene for fruit-ripening.

An outstanding feature of the book is Table 2, which occupies no fewer than 70 of the 326 pages. It lists the species and varieties of cultivated plants in which hormone treatment of cuttings has proved advantageous, and should be of great value to those concerned with plant propagation. Those unfamiliar with the subject, however, may be rather puzzled to find that some of the species mentioned (e.g. Hop, var. Brewer's Gold) occur also in Table 3 under the heading 'Cuttings of plants reported *not* to respond to hormone treatments tested'. It is hoped that in subsequent editions it will be found possible to include in Chapter VIII a further table giving a list of weed species which have proved susceptible to hormone treatment, and to include the scientific names of such weeds as Crabgrass (p. 220) and Chokeberry (p. 226) which will be unfamiliar to non-American readers.

In spite of the publisher's warning about post-war shortages, the general level of production and illustration appear to be very good, and, apart from a curious error in the page headings of Chapter IV, misprints are almost non-existent. It is undoubtedly a book which should find an immediate place in the library of all those interested in the practice and science of horticulture.

L. C. LUCKWILL